BATS AND HUMAN HEALTH

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Ebola, SARS, Rabies and Beyond

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FOREWORD

A BRIEF INTRODUCTION TO UNIQUE FEATURES OF BATS IN RELATION TO INFECTIOUS DISEASES

Over the course of the past half century, a multitude of infectious diseases have come to the attention of the research and health communities. These infectious "emerging diseases" are composed of not only new human diseases or diseases of which we are newly aware, but also include some older infectious diseases that are increasing in virulence or in geographical locations. Emerging infections may be caused by bacteria, viruses, fungi, protozoa, or parasitic worms. The rate of emergence has been increasing and appears to be due to a combination of increased detection and recognition as well as increased numbers of microbial pathogens. It should be noted that we are also experiencing an increase in emerging diseases that are not of microbial origin and are due partially to increased recognition, but also due to changes in our lifestyles, to increased lifespans, and to the rescue of populations of people who previously would not have survived fetal development, infancy, or childhood. A few of these noninfectious emerging diseases include a variety of cancers, obesity-related disorders, and neurological and developmental illnesses, but also fibromyalgia, systemic lupus erythematosus (lupus), temporomandibular joint disorder, a wide range of autoimmune diseases, and carpal tunnel syndrome. Furthermore, many older diseases of infectious origin are increasingly less common due to the efforts of the biomedical research community and health-care professionals and include the "childhood diseases," smallpox, polio, malaria, and rheumatic fever (resulting from immune responses to streptococcal infection), as well as cholera and diarrheal and respiratory diseases in developed areas of the world.

Other than increased detection, a number of factors contribute to the emergence of infectious diseases in human populations. For zoonotic diseases, these include increased contact with microbial reservoir hosts by elimination of their natural habitat plus the related urbanization of many animal species, increased numbers of humans traveling to or residing in formerly lightly inhabited regions, increased contact between previously separated animal species in live animal markets, and the movement of agricultural or companion areas throughout the world.

Bats have several characteristics that combine to make them uniquely qualified to serve as viral hosts. These characteristics are discussed in detail in several journal articles, reviews, and books (Omatsu *et al*. 2007; Wang *et al*. 2011; Hayman *et al*. 2013; Smith & Wang 2013; O'Shea *et al*. 2014; Racey 2015) and so will be mentioned only briefly here. Bats are among the largest and most diverse groups of mammals, second only to rodents. Bats are the only mammals capable of true flight. Large nightly increases in body temperature and energy use required by flight alternate with decreases in temperature and energy

usage occurring during their daily torpor. This increase in body temperature is similar to the fever response and may select for viruses that are able to survive if transmitted to another mammalian host. Bats are known to host a number of viruses that do not cause serious disease in them, supporting the contention that bats are ideal reservoir hosts for many viruses. Lyssaviruses are the most important exception to nonpathogenic viruses of bats. Understanding the mechanisms behind lyssaviruses survival and pathogenicity in bats requires further study into the ways in which this group of viruses differs from other rhabdoviruses which are less pathogenic to bats. It is also very important to determine whether highly pathogenic viruses in humans which have been linked to zoonotic transmission from bats (the Ebola and Marburg filoviruses, henipaviruses, and the SARS- and MERS-coronavirus), are more resistant to higher temperatures *in vitro*. In the case of filoviruses, this might indeed be the case since they cause hemorrhagic fever in humans.

Bat antiviral immune responses differ from those utilized by humans, with bats relying more heavily on protection by interferons, some of which are constitutively expressed (innate immune response) (Zhou *et al*. 2016), rather than the primary human reliance upon CD8+ T killer cells (adaptive immune response) and natural killer cells. This difference, as well as decreased immunity during hibernation in some species of temperate bats, has led to the suggestion that bats are able to control pathogenic viral activity while not clearing the infection, thus maintaining a state of persistent infection, as would be expected of a viral reservoir host. Many bats are long-lived and many species are gregarious and roost in colonies that are composed of over a million bats, sometimes of different species. This facilitates both intraspecies and interspecies horizontal transfer of viruses. Vertical transfer of viruses occurs as well, allowing viruses to persist within colonies long-term. Long distance migration in some bat species also allows wide geographical spread of infection.

While a large amount of attention has focused upon the potential roles of bats, rodents, and nonhuman primates as major reservoirs of emerging viral infections, many other animal species are responsible for direct or indirect zoonotic infection of humans by acting as either reservoir hosts or microbial vectors, as described in Chapter 15. This relatively limited focus on selective animal groups may be a double-edged sword that, while detecting zoonotic reservoir host species, may also miss many other reservoir species. This approach may also focus on viruses of the targeted mammal populations that are similar to those causing disease in humans, but are unlikely to ever live up to their zoonotic potential. The focus on bats and rodents as potential disease reservoirs has also led to fear in the general public and killing or dispersing animal species that humans historically have viewed with fear and loathing. This misguided and generalized fear of bats further decreases the chance of survival for bat species that were already endangered by human activities, including the spread of white-nose syndrome and construction of wind farms (Erickson *et al*. 2016).

The fear of bat-borne diseases and of bats in general overlooks the vital role that bats play, not only in nature, but also in human health and well-being. Bats are major pollinators that are necessary to the continued survival of some plant species, including agave, a key economical crop in regions of Latin America. By consuming insects, some bat species also remove huge numbers of pests that consume crops, reducing the levels of toxic insecticides needed by the agricultural community, and delaying the development of pesticide resistance (reviewed by McCracken *et al*. 2012). Some insectivorous bats eat the equivalent of half their body weight per night and have been estimated to lower agriculture costs by billions of dollars per year in the United States (Hill & Smith 1992; Boyles *et al*. 2011).

Their role in crop protection increases food production in areas of the world which cannot afford inorganic fertilizers. In addition to consuming insect pests, bat guano is used as organic fertilizer. Sale of bat guano is an important part of local economies in many parts of the world. Bats also play critical roles in the repopulation of ecosystems by distributing seeds to damaged areas.

While the majority of scrutiny on bat microbes has focused on viral diseases, bats, as well as other mammals, are infected by many other infectious agents. The increased attention on diseases of bats could, and perhaps should, be extended to other groups of microbes. A better understanding of the microbiome of bats could aid in conservation efforts as we better understand the microbes that threaten bats' well-being. The purpose of this book is to gather known information about microbes infecting bats and discuss their implications for human and bat health. As an aid to study this collection of the microbes that infect bats, spreadsheets containing information about the bat microbes for each chapter that may be easily manipulated for research purposes are found in the companion website. The companion site also contains a master spread-sheet that encompasses information from the chapter spread-sheets as well as including information concerning the bats' diets and geographical locations and further information about the respective microbes. It is hoped that these spread-sheets may be of benefit to not only those who study bat and human infections, but also to the bat conservation community as microbial threats to bats are better understood.

REFERENCES

Boyles JG, Cryan PM, McCracken GF, Kunz TH. 2011. Economic importance of bats in agriculture. *Science.* 332:41–42.

Erickson RA, Thogmartin WE, Diffendorfer JE, Russell RE, Szymanski JA. 2016. Effects of wind energy generation and white-nose syndrome on the viability of the Indiana bat. *PeerJ.* 4:e2830.

Hayman DTS, Bowen RA, Cryan PM, McCracken GF, O'Shea TJ, Peel AJ, Gilbert A, Webb CT, Wood JLM. 2013. Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. *Zoonoses and Public Health.* 60:2–21.

Hill JE, Smith JD. 1992. *Bats: A Natural History*. University of Texas Press: Austin, TX.

McCracken GF, Westbrook JK, Brown VA, Eldridge M, Federico P, Kunz TH. 2012. Bats track and exploit changes in insect pest populations. *PLoS ONE.* 7(8):e43839.

Omatsu T, Watanabe S, Akashi H, Yoshikawa Y. 2007. Biological characters of bats in relation to natural reservoir of emerging viruses. *Comparative Immunology, Microbiology & Infectious Diseases.* 30:357–374.

O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DTS, Luis AD, Peel AJ, Plowright RK, Wood JLN. 2014. Bat flight and zoonotic viruses. *Emerging Infectious Diseases.* 20(5):741–745.

Racey PA. 2015. The uniqueness of bats. In: *Bats and Viruses: A New Frontier of Emerging Infectious Diseases*. L-F Wang and C Cowled (eds). Wiley Blackwell: Hoboken, NJ, pp. 1–22.

Smith I, Wang L-F. 2013. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Current Opinion in Virology.* 3:84–91.

Wang L-F, Walker PJ, Poon LLM. 2011. Mass extinctions, biodiversity and mitochondrial function: are bats 'special' as reservoirs for emerging viruses? *Current Opinion in Virology.* 1:649–657.

Zhou P, Tachedjian M, Wynne JW, Boyd V, Cui J, Smith I, Cowled C, Ng JHJ, Mok L, Michalski WP, Mendenhall IH, Tachedjian G, Wang L-F, Baker ML. 2016. Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. *PNAS.* 113(10):2696–2701.

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I

INTRODUCTION

1

BAT IMMUNOLOGY

1.1 INTRODUCTION TO THE IMMUNE SYSTEM OF BATS

A number of studies have explored the bat immune system in order to determine its components and their activity levels. Bats possess immunocompetent organs and cells similar to those in humans and mice, including the thymus, bone marrow, spleen, lymph nodes, neutrophils, T and B lymphocytes, monocyte/macrophages, eosinophils, basophils, and follicular dendritic cells. These leukocytes (white blood cells) are found in ratios similar to those in mice. They mount a delayed and somewhat smaller humoral and cell-mediated immune response than mice (Paul & Chakravarty 1986; Sarkar & Chakravarty 1991; Schinnerl *et al*. 2011). Regulatory T lymphocytes which dampen the immune response appear to be responsible for the delay (Chakravarty & Paul 1987). Another notable difference between bats and terrestrial mammals is the loss of AIM2 and IFI16 genes which sense microbial DNA, perhaps reducing bat sensitivity to bacteria (Stockmaier *et al*. 2015).

1.1.1 White blood cell count and other serological parameters

White blood cell (WBC) numbers in *Saccopteryx bilineata* (greater sac-winged bat) decrease with age within individuals. IgG antibody levels, however, are higher in older bats. Individuals of this bat species that have higher WBC counts or IgG concentrations had a lower chance to survive the next 6 months (Schneeberger *et al*. 2014). Energetically costly immunological responses are traded against other costly life activities, leading to a reduction in overall lifespan. Immune-mediated generation of pro-oxidants may contribute to this

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reduction. In the neotropical fruit bat, *Carollia perspicillata*, WBC numbers correlate with indicators of oxidative stress (Schneeberger *et al*. 2013a). Interestingly, infection with trypanosomes or nematodes does not correlate with higher WBC counts, IgG concentrations, or survival.

Among 26 species of neotropical bats, the total WBC counts were lower for insectivorous emballonurid, molossid, and vespertilionid bat species than for plant-eating phyllostomid bats, with *Ectophylla alba* (Phyllostomidae), being less than half of that of all other bat species examined (range = 1714 ± 297/μl for *Molossus bondae* to 7339 ± 1503/μl for *Trachops cirrhosis*) (Schinnerl *et al*. 2011). The insectivorous diet, with its higher energy demands, may be at least partially responsible for the decreased WBC numbers. Many of the lymphocytes have an indented nucleus and cytoplasmatic granules, unlike humans, whose lymphocytes have round nuclei and are agranular. Bats, in general, also have higher than normal red blood cell count, hematocrit values, and hemoglobin concentrations than most mammals, perhaps due to the great energy expenditure and aerobic respiration activity and, therefore, oxygen levels, required for flight. Accordingly, total WBC count inversely correlates with hematocrit values. The highest hematocrit levels were found in *M. bondae* and *Molossus sinaloae* (Schinnerl *et al*. 2011). Additionally, polychromatophilic erythrocytes (young red blood cells) levels were high in these animals.

Among wild-caught, healthy Indian flying foxes (*Pteropus giganteus*), the mean lymphocyte differential count is higher for juveniles than adults. Plasma biochemistry, however, is similar between males and females, juveniles and adults, and lactating and nonlactating females. Blood urea nitrogen and cholesterol concentrations are lower in *P. giganteus* than in other tested mammalian groups, but correspond with that seen in other *Pteropus* species. Alanine aminotransferase and AST levels, however, are higher than those reported for closely related *Pteropus vampyrus* (McLaughlin *et al*. 2007).

When Pallas's mastiff bats (*Molossus molossus*) are administered lipopolysaccharide (LPS), an immune system agonist, in order to study their acute phase reactions, they lose body mass. Unlike other LPS-stimulated mammals, however, they do not develop either leucocytosis or fever. During flight on a daily basis, bats' internal body temperature rises to 40°C, mimicking fever. LPS also does not affect the subsequent energy-conserving reduction in temperature, down to approximately 28°C, which occurs during torpor (O'Shea *et al*. 2014; Stockmaier *et al*. 2015).

1.1.2 Innate versus adaptive immunity

Active adaptive immune system activity consumes a great deal of energy that could be used for other essential activities, such as mating and reproduction, as well as longevity. Innate immunity tends to require lower energy expenditure than cell-mediated or adaptive immunity, suggesting that bat species may differ from other mammals in the type and amount of innate versus adaptive immune responses, with an increased reliance upon the former (Schneeberger *et al*. 2013b). Innate immunity also is more rapid than adaptive immunity, perhaps allowing bats to clear viral infections earlier than occurs in humans (Baker & Zhou 2015).

The swelling induced by the phytohemagglutinin skin test is used to measure delayed-type cellular activity of the adaptive immune response. In the Brazilian free-tailed bat (*Tadarida brasiliensis*), this test revealed an early peak of lymphocyte influx, followed by a later peak in infiltrating neutrophils, as well as a high degree of intraspecies variation.

Host roosting ecology, diet, life history, pathogen exposure, and age may contribute to this variation (Turmelle *et al*. 2010). Adaptive immune responses of bat species also vary with body mass.

Bactericidal activity of whole blood utilizes phagocytosis by neutrophils and complement-mediated cytotoxicity of the innate immune response, both of which are important in defense against, and rapid responses to, infection. The subsequent onset of adaptive T cell-mediated immunity is more important in clearance of bacterial infections than in preventing infection (Allen *et al*. 2009). In *T. brasiliensis*, bactericidal activity negatively correlated with shelter permanence. While significant immune activity varies among individuals, colony-level effects also play a role in the extent of bactericidal activity. Females roosting at one cave had lower blood bactericidal activity than blood from females at three other sites, whether caves or bridges. It would be interesting to study whether the bactericidal levels are constant within a given roost or vary with time as the colony faces different bacterial or viral threats.

T cell-mediated immunity is also associated with roost location, as females from two caves had higher responses than females roosting in two bridges. Animals roosting in caves also bear a higher ectoparasite presence, since females in the cave with the lowest blood bactericidal activity also carry a greater burden of mites. Both T cell-mediated immunity and bactericidal activity show negative correlation on the individual level (Allen *et al*. 2009). *T. brasiliensis* maternity roosts form very large colonies, ranging from several thousand to several million individuals in caves and under highway bridges. This type of roosting ecology allows increased exposure to pathogens, with the resulting effects shaping immune defenses. Such a relationship between colonial living and immune responsiveness has also been reported in several avian species (Allen *et al*. 2009).

1.1.3 MicroRNA

Deep sequencing of the small RNA transcriptome of the black flying fox (*Pteropus alecto*) detected 399 microRNAs, of which more than 100 are unique among vertebrates. MicroRNAs are important negative regulators of eukaryotic gene expression. Clusters of rapidly evolving microRNAs appear to target genes regulating virus–host interaction in bats by dampening inflammatory responses, thus limiting immunopathology and possibly energy expenditure as well. Such genes include those active in antiviral immunity, DNA damage response, apoptosis, and autophagy. Understanding the roles of these microRNAs is important since *P. alecto* may be a natural reservoir of the human pathogens Hendra virus and Australian bat lyssavirus (Cowled *et al*. 2014). MicroRNAs have also been identified in the little brown bat (*Myotis lucifugus*), the big brown bat (*Eptesicus fuscus*), and the Jamaican flying fox (*Artebius jamaicensis*) (reviewed by Cowled *et al*. 2014).

1.2 VIRAL PATTERN-RECOGNITION RECEPTORS AND THE BAT IMMUNE RESPONSE TO MICROBES

Molecular patterns used by the host to recognize viral infections are more limited than those used to recognize bacteria and commonly consist of nucleic acid recognition. Viral DNA and RNA are detected by several different classes of host pattern-recognition

receptors, such as retinoic acid inducible gene I (RIG-I)-like receptors (RLRs) in the cytoplasm, Toll-like receptors (TLRs), NOD-like receptors (NLRs), and the cyclic GMP-AMP synthase (cGAS) and 20-50-oligoadenylate synthetase (OAS) nucleotidyltransferases.

TLRs 3, 7, 8 and 9 are found in endosomes and detect dsRNA after endocytosis. TLRs 3, 7, 8 recognize viral RNA, while TLR 9 recognizes viral, bacterial and protozoan DNA. TLRs' ligand recognition properties vary among species. Bat TLRs 3, 7, 8, and 9, in general, evolved under similar functional constraints as other mammals and those of *Desmoids rotundus* display the classic genetic characteristics and three-dimensional structure seen in other mammals (Escalera-Zamudio *et al*. 2015). TLR 9 of bats, however, form a monophyletic clade positioned externally to all other eutherian mammals. Comparison of TLR among eight bat species revealed that TLR evolution in bats is order-specific. This may reflect the need of different bat groups to adapt to a wide variety of ecological niches containing different pathogens profiles. While most bat-specific mutations of the ligand-binding site are unlikely to alter their function, some unique, nonconservative mutations are also present in the ligand-binding sites of bat TLR 9 that might influence its ligand-binding specificity. The adaptations found in the TLRs among bat groups and between bats and other mammalian TLRs may aid in resistance to infection by specific pathogens found in different environments (Escalera-Zamudio *et al*. 2015).

TLRs 1, 2, 4, 5, 6, and 11 are expressed on the cell surface and recognize protein, lipid, and carbohydrate moieties in bacteria, protozoa, and fungi (Cowled *et al*. 2011). RIG-I-like receptors are cytoplasmic and detect viral RNA generated during their replication. The cytoplasmic cGAS recognizes short pieces of double-stranded DNA and activates the Stimulator of Interferon Genes (STING) in the endoplasmic reticulum. This stimulates expression of type I IFN genes via TBK1-IRF3 (TANK binding kinase 1/interferon response factor 3) signaling. It recognizes DNA viruses and bacterial DNA and as well as some RNA viruses. Three-dimensional X-ray crystal structures of cGAS and OAS1 show considerable similarity, despite the fact that OAS1 recognizes double-stranded RNA and that the proteins have very different DNA sequences (Hancks *et al*. 2015). Binding of OAS and cGAS to double-stranded RNA or double-stranded DNA, respectively, produces nucleotide second messengers that activate RNase L (OAS) and STING (cGAS), initiating antiviral responses. Both of these genes are under positive selection and may undergo parallel evolution (Mozzi *et al*. 2015). Long stretches of unmodified dsRNA, while found in RNA and DNA viruses, are not produced by host cells. Host dsRNA sensors include protein kinase R (PKR), which suppresses viral protein synthesis, and RLR melanoma differentiation-associated gene-5 (MDA-5), which induces interferon production. In addition to its antiviral activities, OASs may also play a role in antibacterial defense and cancer suppression (reviewed by Lohöfener *et al*. 2015). The RIG-I like helicases retinoic acid-inducible protein (RIG-1) and MDA-5 are important cytosolic pattern-recognition receptors that detect viral RNA, with RIG-I recognizing short dsRNA and MDA5 recognizing long dsRNA (Siu *et al*. 2014).

The TLR mRNAs in *P. alecto* and *Rousettus leschenaultia* have been cloned. Genome or transcriptome data also detect TRL in *M. lucifugus* and *Artibeus jamaicensis* (Schountz 2014). *P. alecto* TLR 1 to TLR 10 have a high degree of similarity to those of humans and other mammals. TLR 3, however, is highly expressed in bat liver, unlike the case in other mammals where it is primarily expressed in dendritic cells (Cowled *et al*. 2011). Cowled *et al*. (2012) also cloned the genes for RIG-I, MDA-5, and LGP2 in *P. alecto* and found that their primary structure and tissue expression

patterns are similar to that found in humans. Bat databases also contain genes for the NLR members *Ciita*, *Nod1*, *Nod2* (Schountz 2014).

1.3 INTRODUCTION TO THE INTERFERONS

Humans produce a number of type I IFNs: IFN-α, with 13 subtypes, and IFN-β, in addition to a single gene for IFN-κ, IFN-ε, and IFN-ω (Kepler *et al*. 2010). Bat IFNs are only distantly related to those of humans and other mammals and those from Megachiroptera and Microchiroptera are separated into two genetic groups (He *et al*. 2014). Sixty-one ORF for type I IFNs were found in the bats *M. lucifugus* and *P. vampyrus*. They are divided into several distinct subfamilies, including IFN-α, IFN-β, IFN-κ, IFN-ω, and IFN-δ (Kepler *et al*. 2010). The single type II IFN is IFN-γ (immune interferon), while the type III IFNs are composed of groups of IFN-λ genes. The latter family includes four groups in humans, IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B), and IFN-λ4. Of these, IFN-λ1 and IFN-λ3 genes have been also found in *P. alecto* (reviewed in Virtue *et al*. 2011a). *Dobsonia viridis* contains eight IFN-α gene types (amino acid similarity 88.4–99.4%) plus one pseudogene. Phylogenetic studies which compare the type I IFNs of bats with those of other mammals show that these genes are under positive selection and diversity is due to duplication and gene conversion (He *et al*. 2010).

1.3.1 Regulation of interferon production

Interferon production relies upon a family of nine IFN-response factors (IRFs) in humans, of which only IRF1, IRF3, IRF5 and IRF7 appear to be positive regulators of type I IFN transcription, with IRF3 and IRF7 promoting antiviral activity. IRF7 is the master regulator of type I IFN-dependent, and perhaps also type III-dependent, immune responses. It is constitutively expressed in plasmacytoid dendritic cells, cells of the innate immune response which specialize in IFN production, and at low levels in most other cell types. IRF7 is found in lymphatic tissues while nonimmune tissues express almost undetectable levels unless stimulated by type I IFN (reviewed by J. Zhou *et al*. 2014).

IFN induction in fibroblasts utilizes an intracellular pathway in which dsRNA or 5′-triphosphorylated ssRNA of RNA viruses bind to one of two cellular RNA helicases, MDA-5 or RIG-1, respectively, to phosphorylate IRF3 via TBK-1 or IKKε. Phosphorylated IRF3 forms a homodimer that translocates into the nucleus where it stimulates IFN-β gene expression via the transcriptional coactivators p300 and CREB-binding protein. In order to fully activate the IFN-β promoter, IRF3 acts in concert with the transcription factors NF-κB and AP-1. NF-κB is activated in part by PKR, a protein kinase that also recognizes dsRNA. This first-wave of IFN production triggers expression of IRF7. IRF7 may be activated in the same way as IRF3, stimulating a positive-feedback loop that stimulates production of IFN-α in a second wave (reviewed by Thiel & Weber 2008).

The primary IFN producers of the lymphatic system are myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC). The mDC utilize the intracellular pathway as well as a second, endosomal TLR 3 pathway. Additionally, mDC, as well as monocytes, specifically produce IFN-β, IFNλ1, and IFNλ2. In contrast, pDC use endosomal TLR7 and TLR8 to recognize ssRNA to produce all IFN types (reviewed by Thiel & Weber 2008; Lazear *et al*. 2015). All TLRs except TLR 3 activate IRF7 via the adaptor protein,

MyD88 (myeloid differentiation primary response gene 88). MyD88 forms a complex with the kinases IRAK-4 (interleukin 1 receptor associated kinase 4), IRAK-1 and TRAF-6 (TNF receptor-associated factor), which binds directly to IRF7. This leads to TRAF-6-mediated ubiquitination and IRAK1 or IKK-1(IκB kinase-1)-dependent phosphorylation and nuclear translocation of IRF7. IRF7 then binds promoter elements and induces IFN transcription. The human IFN-β and the IFN-α promoter regions have four or two to three positive regulatory domains, respectively, that are binding sites for IRFs (reviewed by J. Zhou *et al*. 2014).

TLR 3 and TLR 4 activate IRF7 via the adaptor molecule TRIF (TIR-domain-containing adapter-inducing IFN-β) which forms a complex with TBK1, IKK-ε (inhibitor of nuclear factor-κB kinase-ε), and IRF7. The phosphorylated IRF7 forms a homodimer or a heterodimer with IRF3 prior to nuclear translocation and induction of type I or type III IFN (reviewed by J. Zhou *et al*. 2014). A large amount of constitutively expressed IRF7 is found in pDCs and the levels are further upregulated by a positive feedback loop to produce high levels of IFN-α and IFN-β (reviewed by Thiel & Weber 2008).

1.3.2 The JAK-STAT pathway and interferon-stimulated genes

IFN-α/β bind to the type I IFN receptors present on almost all cells. Conformational changes in the intracellular region of the receptor activate the Janus kinase/signal transducer and activator of a transcription (JAK-STAT) signaling pathway. The JAK family members JAK-1 and TYK-2 phosphorylate two STAT proteins (signal transducer and activator of transcription 1), STAT-1 and STAT-2. They form a heterodimer that recruits IRF-9 to form the IFN stimulated gene factor 3 (ISGF-3) complex that translocates to the nucleus where it binds and activates IFN-stimulated response elements (ISRE) in promoter regions of IFN-stimulated genes (ISG) (reviewed by Thiel & Weber 2008).

Some ISG have antiviral activities, including the GTPase Mx1 (orthomyxovirus-resistant gene 1), PKR, and the 2′-5′ oligoadenylate synthetases (2-5 OAS)/RNaseL system. Mx1 protects against infection with many RNA and some DNA viruses by binding and inactivating their ribonucleocapsid. PKR is a serine-threonine kinase that phosphorylates the eukaryotic translation initiation factor eIF2, thus blocking translation of cellular and viral mRNAs. The 2-5 OAS catalyzes synthesis of short 2′-5′ oligoadenylates that induce the latent endoribonuclease RNaseL to degrade viral and cellular RNAs. PKR and OAS/RNaseL eliminate virally infected cells by suicide resulting from reduced basal activity. They are constitutively expressed in an inactive form and are upregulated by type I and type III IFNs. Mx1 is not found in resting cells, but is induced by type I and type III IFNs (reviewed by Zhou *et al*. 2013). The promoter region of human PKR contains conserved KCS (kinase conserved sequence)-ISRE promoter elements, permitting a high degree of PKR induction following IFN stimulation. Additionally, IRF-1 activates PKR in the absence of IFN signaling in stimulated human cells. Human Mx1 and OAS1 also contain ISREs, but, unlike PKR, their induction is highly dependent on IFN signals.

Transcriptome analysis of stimulated immune cells from *P. alecto* detected a number of ISGs including Mx1, Mx2, OAS1, OAS2, OASL and PKR (Zhou *et al*. 2013). The functional domains and promoters of the bat *P. alecto*'s Mx1, PKR, and OAS1 are highly conserved with respect to those of other mammals, but *P. alecto Oas1* has two ISRE in its promoter while the human *Oas1* has only one. This may increase the inducibility of the bat gene by type I and type III IFNs. Bat OAS1 and Mx1 were induced

in a highly IFN-dependent manner after stimulation by IFN or dsRNA, but, as is the case in humans, PKR may be induced by an IFN-independent mechanism.

Pteropine orthoreovirus NB (PRV1NB) (Nelson Bay virus) is a dsRNA reovirus of fruit bats while Sendai virus is a negative-strand RNA paramyxovirus widely used to induce IFN. Bat *Oas1* was most readily induced of these ISGs by IFN stimulation or Sendai, or to a lesser extent PRV1NB, infection. While *Mx1* was inducible by either virus, *Pkr* was barely upregulated at all, nor was it induced by IFN stimulation, as occurs in humans. Bat *Pkr* is induced by the dsRNA analog poly (I:C), a viral-associated molecular pattern which induces type I IFN, however. Due to its greater inducibility, OAS1 may therefore have the major antiviral role at least in this species or group of bats (Zhou *et al*. 2013). Sendai virus also induces a stronger IFN-β and IFN-κ2 response than PRV1NB. Both of these viruses may antagonize PKR responses in bats. Reoviruses have been shown to encode proteins that sequester dsRNA and reduce activation of *Pkr* and *Oas1* by dsRNA. *Oas2* is upregulated in vesicular stomatitis virus-infected *P. vampyrus* immune cells to a greater extent than in poly (I:C)-treated cells (Kepler *et al*. 2010).

Stimulation of *Rousettus aegyptiacus* primary kidney cells with human IFN-α induces phosphorylation and nuclear translocation of STAT-1. As is the case with human cells, infection with rabies virus inhibits nuclear translocation in IFN-stimulated bat cells but not its phosphorylation. *R. aegyptiacus* STAT1 mRNA is highly expressed in the liver and to a low extent in muscle and spleen (Fuiji *et al*. 2010). RIG-I, STAT1, and IFN-β were also cloned and sequenced in *R. sinicus* and *R. affinis* horseshoe bats (Li *et al*. 2015). The *Rhinolophus* RIG-I sequences have 87% nucleotide and 82% amino acid identity to that of humans and the most similarity to that of *P. alecto* (91% nucleotide and 86% amino acid identity). The *Rhinolophus* STAT-1 sequence has 91% nucleotide and 95% amino acid identity to that of humans and the most similarity to that of *R. aegyptiacus* fruit bats (94% nucleotide and 97% amino acid identity). The *Rhinolophus* IFN-β sequence has the greatest difference with other species, having only 74–76% nucleotide and 59–61% amino acid identity to that of humans and the greatest similarity with the *P. vampyrus* and *R. aegyptiacus* fruit bat (81–84% nucleotide and 69–74% amino acid identity) (Li *et al*. 2015). RIG-I, STAT-1, and IFN-β are all highly expressed in bat spleen, lung, and intestines. Poly (I:C) stimulated a 30 000-fold increase in interferon in bat cells and only a several hundred-fold increase in mouse cells (Li *et al*. 2015). Taking these results together, RIG-I and STAT-1 from several species of bats have similar structures and functions to those of humans.

Other ISG in humans include the RNA-specific adenosine deaminase acting on RNA 1 (ADAR 1), the product of ISG56, and ISG20. ADAR 1 deaminates adenosine on dsRNAs to inosine, leading to genomic mutation. ADAR 1 activation is also inhibited by reoviruses (Zhou *et al*. 2013). ISG56 binds the eukaryotic initiation factor 3e subunit of eIF3 to suppress viral RNA translation (reviewed by Thiel & Weber 2008), while IGS20 is a 3′-5′ exonuclease that degrades ssRNA.

Some viruses, including highly pathogenic members of the Flaviviridae, Filoviridae, Rhabdoviridae, Bunyaviridae, and Reoviridae, use acidic endosomal entry pathways to gain access to the host cell's cytoplasm. The human immune system inhibits viral entry via an ISG, the IFN-induced transmembrane protein 3 (IFITM3) (Benfield *et al*. 2015). IFITMs block cytoplasmic entry by blocking fusion of viral and host cell membranes by multimerization and increasing membrane rigidity. Mouse IFITM plays an important role in limiting influenza-induced morbidity and mortality. In bat cells, poly (I:C) up-regulates

IFITM3 expression. When expressed in the A549 cell line, the *M. myotis* IFITM3 orthologue co-localized with transferrin (found in early endosomes) and CD63 (present in late endosomes or multivesicular bodies). It blocked cytoplasmic entry of pseudotyped viruses expressing glycoproteins from rabies, Mokola virus, Lagos bat virus, and West Caucasian bat virus about 100-fold. IFITM3 reduced viral yield mediated by hemagglutinin from multiple types of influenza virus by over 100-fold as well. Virus production was increased by siRNA knockdown of IRITM3 (Benfield *et al*. 2015). In addition to bats, pigs were also shown to express protective IFITM3.

1.3.3 Type I interferons

Type I IFNs act in a direct antiviral capacity, but also inhibit cell proliferation, regulate apoptosis, and modulate adaptive immunity. They are produced by all nucleated mammalian cells and are upregulated early after infection, activating expression of >300 antiviral and immunomodulatory genes (Thiel & Weber 2008). Dendritic cells produce high levels of IFN-α, while epithelial cells, fibroblasts, and neurons initially release IFN-β and later switch to IFN-α.

All known mammalian type I IFN genes are unusual in that they contain no introns (generally a trait of bacterial genes). The types and numbers of functional subtypes of type I IFNs vary between bats and other mammals as well among bat species. IFN-ω has the greatest number of subtypes in bats, 12 intact members for *M. lucifugus* and 18 for *P. vampyrus*. While the IFN-α family is large in humans, *M. lucifugus* has only pseudogenes, while *P. vampyrus* has 7 intact genes (Kepler *et al*. 2010). The IFN-δ family consists of 5 intact genes in *P. vampyrus* and 11 genes in *M. lucifugus*. Pig placenta is the only other tissue found to contain a functional IFN-δ gene, and it is involved in embryonic development in pigs, not in antiviral activity (Kepler *et al*. 2010). The genome of *M. lucifugus* additionally contains 1 complete IFN-β, 2 IFN-ε, and 2 IFN-κ genes and *P. vampyrus* has 1 intact member of each of these genes (Kepler *et al*. 2010).

1.3.3.1 IFN-α and IFN-β Characterization of IFN-α and IFN-β from *Rousettus aegyptiacus* revealed that they are most closely related to those found in swine (72% amino acid identity) (Omatsu *et al*. 2008). The IFN-α ORF contains 562 base pairs and encodes a 187-amino acid protein while the IFN-β ORF is 558 base pairs and encodes a 186-amino acid protein. Stimulation of *Rousettus leschenaulti* primary kidney cells and the Tb-1 Lu bat lung cell lines with poly (I:C) leads to increased transcription of IFN-β in the former, but not the latter, cells. IFN-α gene expression occurs later, in response to the presence of IFN-β. The production of IFN-β is rapid and transient while that of IFN-α is longer-lasting (Omatsu *et al*. 2008). The difference in gene expression could be due to differences in tissue type or may result from the use of primary versus immortalized cell lines.

E. helvum cells react to viral stimulation by a high degree of induction of type I IFN mRNA, IFN protein secretion, and efficient ISG induction. When infected by O'nyong-nyong virus, *E. helvum* strongly induces IFN genes, but this virus still evades the IFN system by a translational block (Biesold *et al*. 2011).

There is a high seroprevalence for Henda virus among Australian fruit bats despite an absence of illness, unlike the high degree of pathogenicity in humans. In humans, henipavirus protein P gene products interfere with IFN-α and IFN-β production via cellular MDA5 and STAT proteins (reviewed by Virtue *et al*. 2011b). Additionally, infection

of human cells with either Hendra or Nipah viruses fails to induce IFN transcription. The same study found that when exogenous IFN was present in henipavirus-infected human cells, ISG transcription was only partially blocked and that the exogenous IFN greatly reduced numbers of infected cells and syncytia. Thus, in humans, henipavirus immune evasion appears to be due to a large degree to failure to produce type I IFN (Virtue *et al*. 2011b).

Since the bat IFN response system is important for protection against adverse effects of other viruses that cause severe human illness, IFN responses may also be responsible for the persistent, nonclinical, Hendra infections of bats. Lung cell lines from *T. brasiliensis* and an interscapular tumor line from *Myotis velifer incautus* are resistant to henipavirus infection (Virtue *et al*. 2011a). However, Hendra or Nipah infection of lung, kidney, and fetal cell lines derived from *P. alecto* does not induce IFN-α or IFN-β expression and expression of IFN-λ is reduced by 50%. IFN signaling is also antagonized in these cell lines since ISG54 and ISG56 transcription in response to exogenous IFN-α was blocked by henipavirus infection. In these cell lines, therefore, henipavirus infection appears to be controlled by unidentified mechanisms and not by interferon responses (Virtue *et al*. 2011a). It is important to determine whether this is also the case in fetal and adult bat primary cell cultures. Interestingly, in humans, henipavirus infection of human cells inhibits IFN production but not the interferon signaling pathway (Virtue *et al*. 2011b).

Zho *et al*. (2014) have shown the *P. alecto* contains a single, functional, full-length variant of IRF7 that has a wider tissue distribution than that of other mammals. In humans and mice, IRF7 expression is very low in cells other than pDC and cells which are active, while *P. alecto* IRF7 is present in comparable levels in immune-related and nonrelated tissues, including brain, heart, kidney, liver, lung, small intestine, and testis. Stimulation of bat kidney cell lines induces peak levels of IRF7 at 9 h, 3 h later than peaks in bat type I and type III IFNs but similar to that of bat ISGs Mx1, OAS1 and PKR, consistent with IRF7 induction via a type I IFN feedback loop as is seen in other species (P. Zhou *et al*. 2014). Even though the MyD88 binding domain of bat IRF7 has little sequence conservation with that region of human IRF7, the differences do not affect IRF7 function either in IFN transactivation activity or activation by MyD88. Bat IRF7 activates both IFN-α and IFN-β promoters and bat MyD88 and IRF7 have similar binding capacity as those from humans. Deleting the MyD88-binding region of bat IRF7 also decreases IFN activation. Additionally, using siRNA to knockdown IRF7 functions impaired IFN-β induction in Sendai virus-infected cells and enhanced Pulua virus replication (P. Zhou *et al*. 2014).

1.3.3.2 IFN-κ and IFN-ω While the roles of the type I IFNs, IFN-α and IFN-β, are well-known, the importance of IFN-κ and IFN-ω is less well characterized. He *et al*. (2014) found that these genes from brain cell lines of *Eptesicus serotinus* are conserved among most microchiropteran species. Both of their promoters contain transcription factor binding sites typical of mammals, including IRFs, ISREs, and NF-κB. Since differences exist in the various IRFs and positions of IRF and NF-kB binding sites, these genes from *E. serotinus* are likely to have different regulatory mechanisms (He *et al*. 2014). *In vitro*, IFN-ω strongly activates IFN-induced genes and IFN-κ is a weaker activator. IFN-ω also has the stronger anti-lyssaviruses activity in an *E. serotinus* brain cell line, with anti-EBLV-1 activity greater than anti-RABV activity, and the least activity

is directed against EBLV-2. This is relevant since *E. serotinus* is a major host of EBLV-I in Europe (He *et al*. 2014). The situation is more complex, however, since there is a general silencing of IFN-κ, IFN-ω, and their induced genes during infection with bat-associated lyssaviruses, perhaps permitting long-term infection of bats by these viruses.

The IFN-κ gene is found outside the type I IFN genetic locus, suggesting that this gene may undergo independent evolution in different groups of mammals. Indeed, phylogenetic analysis indicates that IFN-κ sequences from Microchiroptera and Megachiroptera group separately from those of other mammals (He *et al*. 2014). While IFN-ω and IFN-κ sequences from *E. serotinus* grouped with those of other Microchiroptera, they are separate from *Myotis* IFNs (*M. lucifugus*, *M. brandtii*, and *M. davidii*). IFN-κ from the Megachiroptera *P. vampyrus* clusters into a nonbat mammalian group (He *et al*. 2014).

1.3.4 Type II interferon

In humans, type II IFN (IFN-γ; immune IFN) is mainly produced by activated T helper 1 cells and constitutively by natural killer (NK) cells. It acts in a paracrine or autocrine manner on macrophages, T cells, and NK cells. IFN II plays a role in the early innate as well as the adaptive immune responses responsible for long-term control of viral infections (reviewed by Janardhana *et al*. 2012). It also stimulates antigen presentation by class I and class II major histocompatibility complex (MHC) molecules and effects cell proliferation and apoptosis via stimulation. Its primary function is not antiviral, although it does repress viral genes and up-regulates host antiviral proteins, such as 2,5-OAS, PKR, guanylate binding protein, and adenosine deaminase (reviewed by Janardhana *et al*. 2012).

IFN-γ from the Hendra virus host, *P. alecto*, is conserved and functionally similar to that of other mammals. *P. alecto* IFN-γ shares 99% amino acid identity with *P. vampyrus* and 70% with *M. lucifugus*, but only 44% similarity with the mouse homolog. The IFN-γ genes *Ifngr1* and *Ifngr2* have been detected in *A. jamaicensis* as well. Features that are conserved with type II IFNs of other species include the proteins' six α helical structure, essential regions in the C-terminal, a high degree of hydrophobicity, and conserved potential N-linked glycosylation sites (Janardhana *et al*. 2012). As is true of other species, mitogen-stimulated *P. alecto* splenocytes secreted IFN-γ, which inhibited viral growth in Semliki Forest virus-infected *P. alecto* kidney cells and the microchiropteran *T. brasiliensis* lung cells. Hendra virus infection of *P. alecto* kidney cells was also inhibited (Janardhana *et al*. 2012).

1.3.5 Type III interferons

The human type III IFNs are the highly conserved IFN-λ1, IFN-λ2, and IFN-λ3. They resemble IL-10 structurally and use the IL-10 receptor as a co-receptor (Lazear *et al*. 2015). IFN-λ receptors in human and rodents are primarily restricted to epithelial cells and differ from those of type I and type II IFNs (Donnelly & Kotenko 2010). While *P. vampyrus* has three IFN-λ genes that are similar to those present in humans, the closely related *P. alecto* appears to only have two functional IFN-λ genes. IFNλ expression is greater in *P. alecto* splenocytes infected with Tioman virus. *Ifit1* recognizes 5′ triphosphate-RNA from single-stranded RNA viruses. IFNλ also inhibited replication of Pulau virus, a dsRNA bat orthoreovirus, and dramatically increased expression of *Ifit1* and, to a lesser extent, *Ddx58* in a *P. alecto* cell line.

These immune molecules have similar antiviral activity to type I and type III IFNs from other mammals. IFNβ and IFNλ trigger expression of the *P. alecto Mx1* gene, a GTPase that may target viral nucleoproteins, and *Oas1*, which activates RNaseL and degradation of viral RNA, but not *Pkr* (Schountz 2014), a GTPase that appears to target viral nucleoproteins.

Bat type III IFNs are differentially induced upon exposure to synthetic dsRNA. Type I and type III IFNs are produced early in infection, with type I induced as early as 30 min and type III IFNs at 1.5 h. Peak expression of both groups occurs at 6 h and decreases by 24 h. IFN-λ2 response to poly (I:C) was approximately100-fold greater than that of IFN-λ1 and expression of IFN-β was higher than either (Zhou *et al*. 2011b). IFN-λ2 may cause as much as a 25-fold induction of ISG56 and 4-fold induction of RIG-I. Type I and type III IFNs utilize different induction pathways, with type I IFN being activated by both endosomal and cytosolic pattern-recognition receptors and type III IFN being activated predominantly by cytosolic molecules such as RIG-I.

Tioman virus is a ssRNA virus belonging to the paramyxovirus family, which includes the henipaviruses, Hendra and Nipah, that infect *P. alecto* and *P. vampyrus*, respectively. The natural bat host of Tioman virus is the closely related *Pteropus hypomelanus*. Type III IFNs are upregulated by infection of bat cell lines by Tioman virus (reviewed in Virtue *et al*. 2011a). In humans and *Pteropus* genera of giant fruit bats, Tioman virus interacts very weakly with STAT2 (Caignard *et al*. 2013), fails to degrade STAT1 in human cells or prevent its nuclear translocation, and is unable to inhibit type I IFN signaling. Tioman virus does, however, bind to human STAT3 and MDA5 and interferes with IL-6 signaling and IFN-β promoter induction in human cells (Caignard *et al*. 2013). Interestingly, while Tioman virus does not upregulate splenic type I IFN production in *P. alecto*, it does induce a type III IFN response (Zhou *et al*. 2011b; Lazear *et al*. 2015). IFN-λ2 is also able to protect *P. alecto* from Pulau virus.

Zhou *et al*. (2011a) cloned and characterized the genes for *P. alecto* IFNλR1 and IL10R2, which compose the type III IFN receptor complex. This complex is functional and has a wide tissue distribution in these bats. Expression of IFNλR1 is greatest in the spleen and small intestine. Epithelial and immune cells are responsive to IFN-λ. Humans produce two splice variants of the IFNλR1 chain, a soluble and truncated transmembrane form. No such alternative splicing of IFNλR1 is present in *P. alecto*. The two splice variants found in humans may negatively regulate IFN-λ and their absence in *P. alecto* may allow for greater IFN-λ activity in at least some bat species.

IFN-λ are believed to be more closely related to the IL-10 cytokine than to type I IFNs, even though they serve as antiviral agents whose biological activities have some overlap with those of type I IFNs, including inducing similar subsets of ISGs. IFN-λ are induced by a variety of viruses, including the human metapneumovirus; respiratory syncytial virus; SARS coronavirus; rotavirus; reovirus; and Sindbis, dengue, vesicular stomatitis, encephalomyocarditis, influenza, hepatitis B, hepatitis C, and Sendai viruses. They play a major role in preventing viral infection via hepatic, respiratory, gastrointestinal, and integumentary epithelia, as well as through the blood:brain barrier.

In response to infection by many viruses, IFN-α amplifies IFN-λ production and IFN-λ also amplifies IFN-α/β production by inducing IRF-1 and IRF-7 (reviewed by Lazear *et al*. 2015). Type III IFNs also suppress T helper 2 responses, increase IFN-γ

production, reduce numbers of T regulatory cells, increase degranulation by $CD8^+$ T killer cells, and attack tumors (Donnelly & Kotenko 2010; Lazear *et al*. 2015).

Viral infection often coinduces type I and type III IFN production by similar pathways, although type III IFN responses are usually the weaker of the two. IFN-λ1 and IFN-β transcription are activated by both IRF3 and IRF7, while IFN-λ2 and IFN-α utilize primarily IRF7. The IFN-λ1 enhanceosome, however, differs from that of IFN-β, suggesting that they are differently regulated and, together, may bypass some of the viral evasive mechanisms, for additional host protection (Stoltz & Klingstrom 2010). When human epithelial cells were infected with Hantaan virus, IFN-λ1 induction preceded that of MxA and IFN-β, and IFN-α was not produced. IFN-λ1 and MxA were also produced in Hantaan virus-infected Vero E6 cells, which do not produce type I IFNs, therefore this virus can induce IFN-λ1 and ISGs without the need for either IFN-α or IFN-β. Activation of IFN-λ1 requires replicating Hantaan virus since inactivated virus did not induce these genes (Stoltz & Klingstrom 2010).

1.3.6 Viral avoidance of the host IFN response

Most disease-causing viruses at least partly block production of IFN-α/β or their downstream mediators. Negative-strand RNA hantaviruses do so by escaping recognition by RIG-I and MDA5, disrupting TBK1-TRAF3 complex formation, or preventing NF-κB nuclear translocation. Host protective responses lead to the production of IFN-λ1, however, which turns on Mx1 (Stoltz & Klingstrom 2010). SARS-CoV and MERS-CV block innate antiviral signaling by blocking type I IFN induction in several cell lines *in vitro* (Matthews *et al*. 2014). MERS-CoV from humans and BtCoV-HKU4 and BtCoV-HKU5 from bats contain accessory proteins that inhibit IFN-β induction in their hosts (Matthews *et al*. 2014). One of their accessory proteins, however, only weakly blocks the NF-κB signaling pathway.

In order to avoid host IFN responses, some viruses block IFN transcription or ISGs. Henipavirus V protein blocks IFN production by sequestering STATs in a cytoplasmic complex that is unable to undergo nuclear translocation (Fujii *et al*. 2010). Upon stimulation *R. aegyptiacus* cells rely on nuclear translocation of phosphorylated STAT1, which bears 96% amino acid similarity to human STAT1. In a bat kidney cell line, rabies virus also inhibits nuclear localization of STAT1 rather than blocking its phosphorylation.

Mapuera virus is a paramyxovirus of the *Rubulavirus* genus that was originally isolated from an asymptomatic *Sturnira lilium* fruit bat in Brazil. Mapuera virus may or may not be pathogenic in humans and its host range is unknown. Mapuera virus V protein serves as a type I IFN antagonist by preventing nuclear translocation of STAT1 and STAT2 following IFN stimulation, without affecting their phosphorylation. Cytoplasmic sequestration blocks formation of the ISGF3 transcription factor complex in cells from diverse mammalian species, including those from bats, humans, monkeys, dogs, horses, and pigs, but not mice. Since some STAT1 is induced in the infected cells, it appears that at least some IFN is being produced. Mapuera virus V protein binds to *mda-5*, but not *rig-1*, and thus inhibits only IFN induction by the former pathway. Other paramyxoviruses have been shown to induce IFN via RIG-I. The antagonism of the IFN pathway in bat and human cells suggests that another protective immune response may be used (Hagmaier *et al*. 2007).

1.4 ANTIBODIES AND B LYMPHOCYTES

Eutherian mammals produce five classes of antibodies: IgG with multiple subclasses, IgM, IgA, IgE, and IgD. Birds, by contrast, lack IgD, IgE, and IgA. Microchiropterians, however, transcribe all five antibody classes, indicating that the restriction of weight required for flight need not alter representation of different antibody classes. Megachiropterans do not produce IgD (Baker & Zhou 2015). An IgG isotype has been detected in *C. perspicillata*, *E. fuscus* has two IgG isotypes, while *M. lucifugus* has five (Bratsch *et al*. 2011). Fruit bats had been found to possess lower levels of antibodies that agglutinate, hemagglutinate, and fix complement upon antigenic stimulation than common laboratory animals. Additionally, antibody production is delayed in these bats (Iha *et al*. 2009).

Antibodies are composed of two identical heavy and two identical light chains, each containing variable (V) and constant (C) regions. The V regions are responsible for recognition of the antibodies' targets (antigens) which initiates a cascade of events, eventually leading to the production and release of highly specific antibodies. The V region is divided into complementary determining regions (CDR) and framework (FW) regions. The three CDR are the regions of the antibody that actually bind antigen, while the FW regions provide structure. The specificity of individual antibodies and the presence of a vast number of microbial and nonmicrobial antigens necessitate a similarly great number of antibodies and a mechanism to allow production of such a large range of diverse antibodies. In contrast to most mammals, one of the primary mechanisms used by primates and rodents to generate antibody diversity is to rearrange regions of antibody genes that encode the variable, antigen-binding component of the antibodies. The V region of antibodies is encoded by one each of multiple, distinct V, D, and J genes. Formation of antibodies involves genetic rearrangement, in which one of the V genes binds to one of the D genes and to one of the J genes to form large numbers of antibodies specific for different antigens.

The variable heavy chain repertoire (VH) is divided into families and three clans. An analysis of the expressed, rearranged antibody VH regions from *P. alecto* and the unarranged repertoire of *P. vampyrus* found that these bats use representative VH genes of families from all three studied VH clans (I, II, III). Most studied mammals, with the exception of primates and rodents, have few or no genes from at least one of the three clans (Baker *et al*. 2010). Pteropid bats also use the same sort of genetic rearrangements of their numerous VH genes and extensive number of D and J gene segments, a higher number than seen in humans. This permits a large number of possible diverse VDJ rearrangements vital for recognition of numerous antigens, including those of microbes. The two studied Pteropid bats, primates, and rodents are the only eutherian species known to have retained a high level of the VH diversity (Baker *et al*. 2010). At least some bats have over 250 germline VH3 genes, 5–15 times greater than that of primates and rodents (Bratsch *et al*. 2011). This should allow a high degree of antibody diversity via VDJ recombination. *M. lucifugus* has indeed been found to have a very high level of diversity of VDJ loci.

One of the key antigen-binding regions of bat antibody variable heavy chain, CDR3, has fewer tyrosine and more arginine in comparison with other animals, perhaps forming antibodies with a greater degree of specificity with a weaker capacity to bind antigen. Bats also have some mutations in the FW3 areas which distinguish them from humans

and mice (Bratsch *et al*. 2011). Bats also fail to produce neutralizing antibody upon infection with some viruses (Baker *et al*. 2010).

In addition to the VDH rearrangements discussed above, many mammals use somatic hypermutation to increase the amount of antibody diversity needed to respond to a large number of antigens. In this process, antibodies undergo a very high rate of mutation in the areas critical to binding antigen. While *M. lucifugus* bats possess a diverse VH gene repertoire which includes five of the seven human VH gene families, they have a very low mutation frequency, decreasing the role of somatic hypermutation in its generation of antibody diversity and indicating a greater reliance on VDJ rearrangements and junctional diversity (ability to rearrange V, D, and J gene segments at more than one site) to generate a highly diverse antibody repertoire (Bratsch *et al*. 2011; Schountz 2014).

B cell activating factor (BAFF) and aproliferation-inducing ligand (APRIL) are members of the proinflammatory tumor necrosis factor (TNF) cytokine family that share two receptors. They are vital to B cell survival and activities, such as B lymphocyte proliferation, maturation, antibody secretion, isotype switching, T cell activation, and T-independent antibody responses. Full-length cDNA of BAFF and APRIL were cloned from the *Vespertilio superans Thomas* bat. They are encoded by 873 and 753 base pair ORFs that encode 290 and 250 amino acids, respectively. Both bat BAFF and APRIL express the typical TNF signature of a transmembrane domain, a putative furin protease cleavage site, and three cysteine residues. BAFF amino acid level identities between bat and dog, horse, human, and mouse are 80.82, 82.76, 77.59, and 55.28%, respectively. APRIL identity with dog, horse, humans, and cattle all exceed 80%. Cloned BAFF and APRIL are functional in that they promote survival and growth of mouse splenic B lymphocytes (You 2012a, 2012b). BAFF expression is high in the spleen and lower in the kidneys and intestine, similar to its localization in humans. APRIL expression is also highest in the spleen but may be found in other tissues, including bone osteoclasts and tumor cells (You 2012b).

Seasonal horizontal transmission of antibodies appears to occur between young bats and adult females. Seronegative bats typically seroconvert to many antigens, including microbial components, at 16–24 months of age, clustering temporally with late pregnancy of adult females (reviewed by Baker *et al*. 2010). Additionally, *Pteropus* and *Myotis* species show seasonal excretion peaks of henipavirus and coronaviruses associated with periods of pregnancy and lactation. Seroconversion in adult males, however, occurs in mid-year (May 2010 and July 2011), close to the April–June mating period in *E. helvum* (Mutere 1968), when aggression among males increases as well as males having more intimate contact with females.

1.5 MACROPHAGES, DENDRITIC CELLS, AND PROINFLAMMATORY CYTOKINES

Mammalian macrophages are typically potent producers of type I IFNs as well as potent pro-inflammatory cytokines, including TNF-α and interleukin (IL)-1 and IL-6. These cytokines have antiviral activity, but are some of the leading causes of immunopathology. There are two types of dendritic cells with different origins and somewhat different roles. Rapid response to viruses or viral components is performed by pDC, which

produce large amounts of type I IFN, have direct antiviral activity, and modulate natural killer cell and CD8 T killer cell activity. While mDC produce large amounts of type I IFN and other immunomodulatory cytokines, they are also antigen-presenting cells which stimulate adaptive immune responses by T lymphocytes.

Monocyte/macrophages play a major role in filovirus pathogenicity in humans, triggering bystander apoptosis of lymphocytes and increased vascular permeability, leading to circulatory collapse. Macrophages also express a cell surface cytokine receptor that interacts with clotting factors VIIa and X to activate the coagulation cascades and the hemorrhagic manifestations of filovirus (reviewed by Basler 2012). Infection of human monocyte/macrophages by filoviruses *in vivo* and *in vitro* induces production of proinflammatory cytokines that attract additional cells to the site which, in turn, also become infected. Dendritic cells are also infected by ebolaviruses but do not produce inflammatory cytokines or initiate T helper cell responses. Interestingly, all of these cells produce little type I or type II IFN (reviewed by Basler). As discussed earlier in this chapter, bats appear to have lesser levels of adaptive immune responses than many other mammals and dampened production of proinflammatory cytokines. This may protect them against the damaging effects of viral infection that lead to life-threatening disease in humans. It should be noted that a relatively small amount of studies has focused on adaptive immunity in bats or their production of proinflammatory or anti-inflammatory cytokines.

1.6 T LYMPHOCYTES

T lymphocyte activity is vital for virus clearance in most viral infections, including coronavirus infections. This has been seen in a Middle Eastern respiratory syndrome coronavirus (MERS-CoV) mouse model and appears to be important in human defense against this virus as well. Immunodominant epitopes which stimulate CD8 T-cells are found in the MERS-CoV S protein (Zhao *et al*. 2014). In humans, severe acute respiratory syndrome (SARS) survivors produce memory T cell responses against the products of the viral S, M, E, NP, and ORF3a genes as well (Oh *et al*. 2011). Six years after recovering from SARS, people still bore SARS-specific memory CD4 T helper lymphocytes and CD8 T killer lymphocytes. Human T memory cells respond primarily to a dominant SARS-CoV nucleocapsid protein by producing and releasing powerful inflammatory mediators, including IFN-γ, TNF-α, and macrophage inflammatory proteins 1α and 1β upon activation by antigen. The $CD4^+$ memory cells produce the Th1 cytokines IFN-γ, TNF-α, and IL-2 (Oh *et al*. 2011). The production of an excessive, detrimental, inflammatory response in humans and the absence of such a reported response in bats may at least partially explain the differences in the pathology of MERS and SARS, and perhaps other viral diseases, in bats and humans.

Recognition and activation of CD4 T helper cells requires interaction between antigens, the T cell receptor, MHC II, and CD4. The complete sequence of *R. aegyptiacus* CD4 cDNA reveals that bat CD4 has more homology to that of cats and dogs than to that of humans and mice. Bats' CD4 Ig-like C-type 1 region contains an insertion of 18 amino acids. Bat CD4, like that of pig, cat, whale, and dog CD4, also lacks a cysteine, an amino acid which forms disulfide bonds and plays a major role in protein folding. Human, monkey, and mouse CD4 have this cysteine, indicating that human and bat CD4 differ in several key structural features (Omatsu *et al*. 2006).

Stressing the importance of CD4 and MHC II contributions to bat population health and fitness, there is a correlation between MHC II *DRB* alleles, hematophagous ectoparasite loads (ticks and bat flies), and the neotropical *Noctilio albiventris* bats' reproductive state. Specific *DRB* alleles are associated with nonreproductive adult males and females, who also bear higher ectoparasite loads than reproductively active animals (Schad *et al*. 2012). The presence of ticks may affect immunity to co-infection with other pathogens since antigen presentation by macrophages and T helper cell functions are reduced by compounds in tick saliva. Only one polyallelic *DRB* gene is found in *N. albiventris*, while two *DRB* gene copies are present in *S. bilineata*. Allelic variation in *S. bilineata* is believed to result primarily from intragenic recombination rather than intergenic recombination (Mayer & Brunner 2009; Schad *et al*. 2012).

The *DRB* gene, especially exon 2, is under positive selection, as evidenced by a greater than 2-fold higher rate of nonsynonymous versus synonymous substitutions, particularly in the antigen-binding sites (Mayer & Brunner 2009; Schad *et al*. 2012). *DRB* is believed to also alter individual bat body odor, as is the case in other mammals. Since bats are an extremely gregarious group of mammals with some colonies containing several million individuals, odor recognition is partially used as a means of recognition. *DRB*, therefore, may also be involved in recognition of family and mate selection (Schad *et al*. 2011) Male *N. albiventris* also have higher heterozygosity rate and genetic variability in the *DRB* gene than do females.

After recognizing antigen presented by MHC class II proteins, T helper lymphocytes produce and secrete cytokines. T lymphocyte-derived cytokine production in bats is delayed in comparison with production by mice. Bat IL-2, IL-4, IL-6, IL-10, IL-12 p40, and TNF-α contain 152, 134, 207, 178, 329, and 232 amino acids, respectively. These genes are highly conserved in comparison with those from horses, dogs, cats, pigs, and cattle. Interestingly, all of these cytokines are encoded by a single exon (Iha *et al*. 2009).

1.7 OTHER PARAMETERS OF THE IMMUNE RESPONSE

Papenfuss *et al*. (2012) explored the transcriptome of *P. alecto* using stimulated spleen, white blood cells, and lymph nodes, in addition to unstimulated thymus and bone marrow. Approximately 18 600 genes were identified. Highly expressed genes were involved in routine cellular processes, such as cell growth and maintenance, enzymatic activity, metabolism, production of cellular components, and energy pathways. Approximately 500 genes, however, were associated with immune function and these composed 3.5% of the transcribed genes in this bat species. The largest proportion of immune genes was associated with T cell activation (79 genes). Other immune-related genes include those involved with natural killer cell cytotoxicity (72), Toll-like receptor cascades (70 genes), B cell activation (50), and antigen presentation (41). Transcriptome analysis also revealed the expression of genes such as pattern-recognition receptors and some, but not all, natural killer cell receptors. Genes for NLRC5 and NLRP3 were also found to be transcribed. NLRC5 is believed to positively and negatively regulate bat antiviral immune responses, while NLRP3 is activated by danger signals, including viral and bacterial infections and environmental irritants. NLRP3 activates caspase-1 in the inflammasome to cleave IL-1β and IL-18 into their mature, active forms (Papenfuss *et al*. 2012).

As would be expected, the transcriptome also included IFN-α and its receptor, as well as genes orthologous to the IFN stimulated genes Mx1, Mx2, OAS1, OAS2, OAS3, OAS-like (OASL), PKR, RNaseL, and ISG15. Natural killer cell-related molecules in the transcriptome include inhibitory CD94/NKG2A, CD24, CD16, and CD56. The MHC class I antigen-loading and presentation pathway in the bat transcriptome include beta-2 microglobulin, transporter associated with antigen processing 1, calnexin, and tapasin, CD1a, CD1b, CD1d, MR1, HFE, FcRn, and ULBPs. The bat MHC class II-associated mRNAs present include homologs to class II invariant (CD74) chain, cathepsin S, the alpha chain homologs of DMA, DOA, DQA, and DRA, and the beta chain homologs of DMB, DOB, DQB, and DRB. Lymphocyte-related molecules found in the transcriptome include α, β, δ, and γ chains of the T cell receptor, the TCRζ chain, CD3, CD4, CD8, and CD28, immunoglobulin variable and constant domains of the heavy and light chains, and B cell co-receptors CD19, CD22, CD72, CD79a, and CD79b.

Transcriptional analysis of *P. vampyrus* bat kidney cells infected with the avian paramyxovirus, Newcastle disease virus, shows that 200–300 antiviral genes are highly upregulated, including genes for IFN-β, RIG-I, MDA5, ISG15, and IRF1. Infection with Hendra and Nipah viruses, by contrast, did not induce these innate immune response genes. Furthermore, the addition of Nipah IFN antagonistic proteins decreased the immune response of the bat kidney cells to Newcastle disease virus (Glennon *et al.* 2015), suggesting that infection by one virus may affect immune responsiveness to other viruses.

1.8 CONCLUSIONS

The immune system of bats and humans are in many ways similar. Bats possess similar immunocompetent organs (thymus, bone marrow, spleen, and lymph nodes) as well as cells responsible for innate (neutrophils, monocyte/macrophages, eosinophils, basophils, and dendritic cells) and adaptive immunity (T and B lymphocytes). They do not, however, possess AIM2 and IFI16 proteins which detect microbial DNA, perhaps increasing bat susceptibility to bacteria. Additionally, the lack of AIM2 may help reduce deleterious inflammatory responses in bats due to its role as one of the molecules that activate the inflammasome. WBC numbers decrease with age in some bat species, while IgG levels increase. Interestingly, in some species, high WBC count or IgG levels may decrease the bat's life-span since the cost of immunological reactivity is balanced with other high energy activities, such as flight. Accordingly, insectivorous bats tend to have lower WBC levels than bats with other diet based upon stationary objects. Production of detrimental reactive oxygen and nitrogen species by neutrophils and macrophages may play a role in the reduced life-span of bats with higher WBC counts. Reactive oxygen species are also generated during aerobic respiration, which is increased in bats due to their large level of energy expenditure. Many bats have relatively high red blood cell counts, hematocrit values, and hemoglobin concentrations that may again be tied to their need for large amounts of energy. Exposure to an immune system agonist decreases bats' body mass, but, unlike other mammals, they do not develop fever. Daily alteration of flight and torpor results in increases and decreases in internal body temperature, respectively, and may impact their microbiomes, resulting in intra- and interspecies microbial variation.

The adaptive immune system requires greater energy expenditure than innate immunity, perhaps promoting bats to rely more heavily on IFN-mediated anti-viral defenses than on cell-mediated activity of $CD8^+$ T killer cells and natural killer cells, as is the case in humans. However, the extent of the role of interferons in bats' antiviral defense may be skewed since relatively little research has focused on cell-mediated immunity in bats.

Neutrophil's phagocytic activity combined with complement-mediated cytotoxicity are rapid and are involved in defense against bacterial infection, while T cell activity is more important in clearing viral infections. Size and location of roosts affect antibacterial immune responses. T cell-mediated immunity and bactericidal activity are negatively correlated.

Pattern-recognition receptors permit the host immune system to recognize and respond to microbes. Most of bats' endosomal TLRs (3, 7, and 8) recognize viral RNA and share genetic characteristics and structure with those from other mammals. TLR 9, however, which recognizes viral, bacterial, and protozoan DNA, is in a monophyletic clade positioned externally to other eutherian mammals. The cell surface TLRs recognize protein, lipid, and carbohydrate from bacteria, protozoa, and fungi. Other host pattern-recognition receptors include RIG-I-like receptors, NOD-like receptors, cGAS, OAS, PKR, and MDA-5. The structure and tissue expression patterns of pattern-recognition receptors in bats are similar to those present in humans.

Bat IFNs are a vital component of their antiviral defenses. There are many more types of type I IFNs in bats than in humans and they are only distantly related. Megachiroptera and Microchiroptera IFNs have additionally been placed into two genetic groups. Numbers of subtypes of type I IFNs differ among bat species, but bats contain more members of the IFN-ω and IFN-δ subtypes than humans and generally fewer IFN-α family members. Bat IFN-κ also fall into a separate phylogenetic group from those of other mammals. As is the case with humans, IFN-α induces phosphorylation and nuclear translocation of STAT-1 and either or both of these processes is blocked by some viruses. Bats contain several ISGs including Mx1, Mx2, OAS1, OAS2, OASL, PKR, and IFITM3. Four groups of type III IFNs are found in humans: IFN-λ1 and IFN-λ3 are also present in at least some bat species. Human IFNλR1 has two alternative splice forms that may negatively regulate IFN-λ. Since at least some bats only have one splice form, they may have greater IFN-λ activity. Macrophages and dendritic cells produce large amounts of type I IFNs. The inflammatory cytokines produced by these cells in humans have antiviral activity, but may also contribute to immunopathology.

Bats produce the same five antibody classes as humans do, however, the number of IgG isotypes varies among bat species. Bats antibodies have more diversity than human antibodies in their variable regions, possessing 5–15 times more VH3 genes than primates or rodents. Unlike humans, bats do not utilize somatic hypermutation to increase antibody diversity.

CD4 T helper cells, a part of the adaptive immune response, are vital to human defenses against microbes and cancerous cells, as evidenced by the severe loss of antimicrobial defenses seen in HIV-positive people upon viral destruction of their T helper cells below a critical threshold. Much less is known about the importance of T lymphocyte function of bats. Interestingly, the CD4 molecule in *R. aegyptiacus* differs from that of humans in several ways, including an 18-amino acid insertion and the lack of a cysteine. MHC II molecules present on antigen presenting cells bind to CD4 during the

initiation of T helper cell activation. One of the polyallelic MCH II molecules, DRB, has been studied in a limited number of bat species. DRB allele usage correlates with bat reproductive state and ectoparasite load. T helper cell functioning is, in turn, affected by ectoparasite saliva. The *DRB* gene is under positive selection and, in addition to its contribution to T helper cell responses, appears to also affect bat odor and recognition of other bats. Bat RNAs for many pro- and anti-inflammatory cytokines are present in the transcriptosomes and are conserved with those from other mammals. These cytokines include IL-2, IL-4, IL-6, IL-10, IL-12 p40, and TNF-α, although the knowledge concerning the actual blood and tissue protein levels is, to a large degree, lacking.

Even though transcriptome analysis found that 3.5% of *P. alecto* genes from stimulated immune organs are associated with immune function, including homologs of mammalian molecules involved in antigen presentation and T and B lymphocyte activation and functioning, natural killer cell cytotoxicity, Toll-like receptor cascades, and components of the IFN systems, very little is known about the adaptive immune response of bats. This is especially true for the T helper and CD8 T killer cells, critical components of human immunity to microbes as well as immunopathological responses. Much more work needs to be done in order to truly understand the similarities and differences of human and bat immune responses to microbes and to cancer. Until this work is well underway and involves studies of numerous species of a diverse range of bats, it will be difficult to draw any clear conclusions about bats' defenses against viral, bacterial, fungal, or parasitic infections or to compare them with defenses utilized by other mammal species.

REFERENCES

Allen LC, Turmelle AS, Mendonc MT, Navara KJ, Kunz TH, McCracken GF. 2009. Roosting ecology and variation in adaptive and innate immune system function in the Brazilian free-tailed bat (*Tadarida brasiliensis*). *Journal of Comparative Physiology B.* 179:315–323.

Baker ML, Tachedjian M, Wang L-F. 2010. Immunoglobulin heavy chain diversity in Pteropid bats: evidence for a diverse and highly specific antigen binding repertoire. *Immunogenetics.* 62:173–184.

Baker ML, Zhou P. 2015. Bat immunology. In: *Bats and Viruses: A New Frontier of Emerging Infectious Diseases*. L-F Wang and C Cowled (eds). Wiley Blackwell: Hoboken, NJ, pp. 327–438.

Basler CF. 2012. Nipah and Hendra virus interactions with the innate immune system. *Current Topics in Microbiology and Immunology.* 359:123–152.

Benfield CTO, Smith SE, Wright E, Wash RS, Ferrara F, Temperton NJ, Kellam P. 2015. Bat and pig IFN-induced transmembrane protein 3 restrict cell entry by influenza virus and lyssaviruses. *Journal of General Virology.* 96:991–1005.

Biesold SE, Ritz D, Gloza-Rausch F, Wollny R, Drexler JF, Corman VM, Kalko EKV, Oppong S, Drosten C, Müller MA. 2011. Type I interferon reaction to viral infection in interferon-competent, immortalized cell lines from the African fruit bat *Eidolon helvum*. *PLoS ONE.* 6(11):e28131.

Bratsch S, Wertz N, Chaloner K, Kunz TH, Butler JE. 2011. The little brown bat, *M. lucifugus*, displays a highly diverse VH, DH and JH repertoire but little evidence of somatic hypermutation. *Developmental and Comparative Immunology*. 35:421–430.

Caignard G, Lucas-Hourani M, Dhondt KP, Labernardiére J-L, Petit T, Jacob Y, Horvat B, Tangy F, Vidalain P-O. 2013. The V protein of Tioman virus is incapable of blocking type I interferon signaling in human cells. *PLoS ONE.* 8(1):e53881.

Chakravarty AK, Paul BN. 1987. Analysis of suppressor factor in delayed immune responses of a bat, *Pteropus giganteus*. *Developmental and Comparative Immunology.* 11(3):649–660.

Cowled C, Baker M, Tachedjian M, Zhou P, Bulach D, Wang L-F. 2011. Molecular characterisation of Toll-like receptors in the black flying fox *Pteropus alecto*. *Developmental and Comparative Immunology.* 35:7–18.

Cowled C, Baker ML, Zhou P, Tachedjian M, Wang L-F. 2012. Molecular characterisation of RIG-I-like helicases in the black flying fox, *Pteropus alecto. Developmental and Comparative Immunology.* 36:657–664.

Cowled C, Stewart CR, Likic VA, Friedländer MR, Tachedjian M, Jenkins KA, Tizard ML, Cottee P, Marsh GA, Zhou P, Baker ML, Bean AG, Wang L-F. 2014. Characterisation of novel microRNAs in the black flying fox (*Pteropus alecto*) by deep sequencing. *BMC Genomics.* 15:682.

Donnelly RP, Kotenko SV. 2010. Interferon-Lambda: a new addition to an old family. *Journal of Interferon & Cytokine Research.* 30(8):555–564.

Escalera-Zamudio M, Zepeda-Mendoza ML, Loza-Rubio E, Rojas-Anaya E, Méndez-Ojeda ML, Arias CF, Greenwood AD. 2015. The evolution of bat nucleic acid-sensing Toll-like receptors. *Molecular Ecology.* 24:5899–5909.

Fujii H, Watanabe S, Yamane D, Ueda N, Iha K, Taniguchi S, Kato K, Tohya Y, Kyuwa S, Yoshikawa Y, Akashi H. 2010. Functional analysis of Rousettus aegyptiacus "signal transducer and activator of transcription 1" (STAT1). *Developmental and Comparative Immunology.* 34:598–602.

Glennon NB, Jabado O, Lo MK, Shaw ML. 2015. Transcriptome profiling of the virus-induced innate immune response in *Pteropus vampyrus* and its attenuation by Nipah virus interferon antagonist functions. *Journal of Virology.* 89(15):7550–7566.

Hancks DC, Hartley MK, Hagan C, Clark NL, Elde NC. 2015. Overlapping patterns of rapid evolution in the nucleic acid sensors cGAS and OAS1 suggest a common mechanism of pathogen antagonism and escape. *PLoS Genetics.* 11(5):e1005203.

Hagmaier K, Stock N, Precious B, Childs K, Wang L-F, Goodbourn S, Randall RE. 2007. Mapuera virus, a rubulavirus that inhibits interferon signaling in a wide variety of mammalian cells without degrading STATs. *Journal of General Virology.* 88:956–966.

He G, He B, Racey PA, Cui J. 2010. Positive selection of the bat interferon alpha gene family. *Biochemistry and Genetics.* 48:840–846.

He X, Korytář T, Schatz J, Freuling CM, Müller T, Köllner B. 2014. Anti-Lyssaviral activity of interferons κ and ω from the serotine bat, *Eptesicus serotinus*. *Journal of Virology.* 8(10):5444–5454.

Iha K, Omatsu T, Watanabe S, Ueda N, Taniguchi S, Fujii H, Ishii Y, Kyuwa S, Akashi H, Yoshikawa Y. 2009. Molecular cloning and sequencing of the cDNAs encoding the bat interleukin (IL)-2, IL-4, IL-6, IL-10, IL-12p40, and tumor necrosis factor-alpha. *Journal of Veterinary Medical Science.* 71(12):1691–1695.

Janardhana V, Tachedjian M, Crameri G, Cowled C, Wang L-F, Baker ML. 2012. Cloning, expression and antiviral activity of IFN-γ from the Australian fruit bat, *Pteropus alecto. Developmental and Comparative Immunology.* 36:610–618.

Kepler TB, Sample C, Hudak K, Roach J, Haines A, Walsh A, Ramsburg EA. 2010. Chiropteran types I and II interferon genes inferred from genome sequencing traces by a statistical gene-family assembler. *BMC Genomics.* 11:444.

Lazear HM, Nice TJ, Diamond MS. 2015. Interferon-l: immune functions at barrier surfaces and beyond. *Immunity.* 43:15–28.

Li J, Zhang G, Cheng D, Ren H, Qian M, Du B. 2015. Molecular characterization of RIG-I, STAT-1 and IFN-beta in the horseshoe bat. *Gene.* 561:115–123.

Lohöfener J, Steinke N, Kay-Fedorov P, Baruch P, Nikulin A, Tishchenko S, Manstein DJ, Fedorov R. 2015. The activation mechanism of 2′-5′-oligoadenylate synthetase gives new insights into OAS/cGAS triggers of innate immunity. *Structure.* 23:851–862.

Matthews KL, Coleman CM, van der Meer Y, Snijder EJ, Frieman MB. 2014. The ORF4b-encoded accessory proteins of Middle East respiratory syndrome coronavirus and two related bat coronaviruses localize to the nucleus and inhibit innate immune signalling. *Journal of General Virology.* 94:874–882.

Mayer F, Brunner A. 2009. Non-neutral evolution of the major histocompatibility complex class II gene *DRB*1in the sac-winged bat *Saccopteryx bilineata*. *Heredity.* 99:257–264.

McLaughlin AB, Epstein JH, Prakash V, Smith CS, Daszak P, Field HE, Cunningham AA. 2007. Plasma biochemistry and hematologic values for wild-caught flying foxes (*Pteropus giganteus*) in India. *Journal of Zoo and Wildlife Medicine.* 38(3):446–452.

Mozzi A, Pontremoli C, Forni D, Clerici M, Pozzoli U, Bresolin N, Cagliani R, Sironi M. 2015. OASes and STING: adaptive evolution in concert. *Genome Biological Evolution.* 7(4):1016–1032.

Mutere FA. 1968. The breeding biology of the fruit bat *Rousettus aegyptiacus E. Geoffroy* living at 0 degrees 22'S. *Acta Tropica.* 25(2):97–108.

Oh H-LJ, Chia A, Chang CXL, Leong HN, Ling KL, Grotenbreg JM, Gehring AJ, Tan YJ, Bertoletti A. 2011. Engineering T cells specific for a dominant severe acute respiratory syndrome coronavirus CD8 T cell epitope. *Journal of Virology.* 85(20):10464–10471.

Omatsu T, Bak E-J, Ishii Y, Kyuwa S, Tohya Y, Akashi H, Yoshikawa Y. 2008. Induction and sequencing of Rousette bat interferon α and β genes. *Veterinary Immunology and Immunopathology.* 124:169–176.

Omatsu T, Nishimura Y, Bak EJ, Ishii Y, Tohya Y, Kyuwa S, Akashi H, Yoshikawa Y. 2006. Molecular cloning and sequencing of the cDNA encoding the bat CD4. *Veterinary Immunology and Immunopathology.* 111:309–313.

O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DT, Luis AD, Peel AJ, Plowright RK, Wood JL. 2014. Bat flight and zoonotic viruses. *Emerging Infectious Diseases.* 20:741–745.

Papenfuss AT, Baker ML, Feng Z-P, Tachedjian M, Crameri G, Cowled C, Ng J, Janardhana V, Field HE, Wang L-F. 2012. The immune gene repertoire of an important viral reservoir, the Australian black flying fox. *BMC Genomics.* 13:261.

Paul BN, Chakravarty AK. 1986. In vitro analysis of delayed immune response in a bat, *Pteropus giganteus*: process of Con-A mediated activation. *Developmental and Comparative Immunology.* 10(1):55–67.

Sarkar SK, Chakravarty AK. 1991. Analysis of immunocompetent cells in the bat, *Pteropus giganteus*: Isolation and scanning electron microscopic characterization. *Developmental and Comparative Immunology.*15(4):423–430.

Schad J, Dechmann DKN, Voigt CC, Sommer S. 2011. MHC class II DRB diversity, selection pattern and population structure in a neotropical bat species, *Noctilio albiventris*. *Heredity.* 107:115–126.

Schad J, Dechmann DKN, Voigt CC, Sommer S. 2012. Evidence for the 'good genes' model: Association of MHC class II *DRB* alleles with ectoparasitism and reproductive state in the neotropical lesser bulldog bat, *Noctilio albiventris*. *PLoS ONE.* 7(5):e37101.

Schinnerl M, Aydinonat D, Schwarzenberger F, Voigt CC. 2011. Hematological survey of common neotropical bat species from Costa Rica. *Journal of Zoo and Wildlife Medicine.* 42(3):382–391.

Schneeberger K, Courtiol A, Czirják GA, Voigt CC. 2014. Immune profile predicts survival and reflects senescence in a small, long-lived mammal, the greater sac-winged bat (*Saccopteryx bilineata*). *PLoS ONE.* 9(9):e108268.

Schneeberger K, Czirják GA, Voigt CC. 2013a. Inflammatory challenge increases measures of oxidative stress in a free-ranging, long-lived mammal. *The Journal of Experimental Biology.* 216:4514–4519.

Schneeberger K, Czirják GA, Voigt CC. 2013b. Measures of the constitutive immune system are linked to diet and roosting habits of neotropical bats. *PLoS ONE.* 8(1):e54023.

Schountz T. 2014. Immunology of bats and their viruses: Challenges and opportunities. *Viruses.* 6:4880–4901.

Siu K-L, Yeung ML, Kok K-H, Yuen K-S, Kew C, Lui P-Y, Chan C-P, Tse H, Woo PCY, Yuen K-Y, Jin D-Y. 2014. Middle East respiratory syndrome coronavirus 4a protein is a double-stranded RNA-binding protein that suppresses PACT-induced activation of RIG-I and MDA5 in the innate antiviral response. *Journal of Virology.* 88(9):4866–4876.

Stockmaier S, Dechmann DKN, Page RA, O'Mara MT. 2015. No fever and leucocytosis in response to a lipopolysaccharide challenge in an insectivorous bat. *Biology Letters.* 11:20150576.

Stoltz M, Klingstrom J. 2010. Alpha/beta interferon (IFN-alpha/beta)-independent induction of IFN-lambda1 (interleukin-29) in response to Hantaan virus infection. *Journal of Virology.* 84:9140–9148.

Thiel V, Weber F. 2008. Interferon and cytokine responses to SARS-coronavirus infection. *Cytokine & Growth Factor Reviews.* 19:121–132.

Turmelle AS, Ellison JA, Mendonc MT, McCracken GF. 2010. Histological assessment of cellular immune response to the phytohemagglutinin skin test in Brazilian free-tailed bats (*Tadarida brasiliensis*). *Journal of Comparative Physiology B.* 180:1155–1164.

Virtue ER, Marsh GA, Baker ML, Wang L-F. 2011a. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS ONE.* 6(7): e22488.

Virtue ER, Marsh GA, Wang LF. 2011b. Interferon signaling remains functional during henipavirus infection of human cell lines. *Journal of Virology.* 85(8):4031–4034.

You F, Ren W, Hou H, Pei L, He Z. 2012a. Molecular cloning, expression, bioinformatics analysis and bioactivity characterization of TNF13B (BAFF) gene in bat (*Vespertilio superans Thomas*). *International Immunopharmacology.* 12:433–440.

You F, Zhou L, Liu X, Fan J, Ke Z, Ren W. 2012b. Molecular structure, expression analysis and functional characterization of APRIL (TNFSF13) gene in bat (*Vespertilio superans Thomas*). *Gene.* 498:196–202.

Zhao J, Li K, Wohlford-Lenane C, Agnihothram SS, Fett C, Zhao J, Gale MJ Jr, Baric RS, Enjuanes L, Gallagher T., McCray PB Jr, Perlman S. 2014. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proceedings of the National Academy of Sciences of the United States of America.* 111(13):4970–4975.

Zhou J, Chu H, Li C, Wong BH, Cheng ZS, Poon VK, Sun T, Lau CC, Wong KK, Chan JY, Chan JF, To KK, Chan KH, Zheng BJ, Yuen KY. 2014. Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. *Journal of Infectious Diseases.* 209:1331–1342.

Zhou P, Cowled C, Mansell A, Monaghan P, Green D, Wu L, Shi Z, Wang L-F, Baker ML. 2014. IRF7 in the Australian black flying fox, *Pteropus alecto*: evidence for a unique expression pattern and functional conservation. *PLoS ONE.* 9(8):e103875.

Zhou P, Cowled C, Marsh GA, Shi Z, Wang L-F, Baker LM. 2011a. Type III IFN receptor expression and functional characterisation in the pteropid bat, *Pteropus alecto*. *PLoS ONE.* 6(9):e25385.

Zhou P, Cowled C, Todd S, Crameri G, Virtue ER, Marsh GA, Klein R, Shi Z, Wang L-F, Baker ML. 2011b. Type III IFNs in pteropid bats: differential expression patterns provide evidence for distinct roles in antiviral immunity. *Journal of Immunology.* 186(5):3138–3147.

Zhou P, Cowled C, Wang L-F, Baker ML. 2013. Bat Mx1 and Oas1, but not Pkr are highly induced by bat interferon and viral infection. *Developmental and Comparative Immunology.* 40:240–247.

II

VIRAL INFECTIONS OF BATS

2

RABIES VIRUS AND OTHER BAT RHABDOVIRUSES

2.1 INTRODUCTION TO THE FAMILY RHABDOVIRIDAE

Rhabdoviridae species are composed of bullet-shaped virions containing a helical nucleocapsid encasing ssRNA (−). They have a wide host range, including vertebrates, invertebrates, and plants. Many rhabdoviruses are transmitted by arthropod vectors, such as mosquitoes, fleas, sandflies, lice, and ticks. The Rhabdoviridae family has a number of genera that include *Cytorhabdovirus, Ephemerovirus, Lyssavirus, Nucleorhabdovirus, Novirhabdovirus, Perhavirus, Sigmavirus, Tibrovirus*, and *Vesiculovirus* in addition to about 150 unassigned rhabdoviruses (reviewed by Ghedin *et al*. 2013). Many species of bats are associated with members of the Rhabdoviridae family (Table 2.1).

2.2 LYSSAVIRUSES

Lyssaviruses cause several severe to fatal diseases in a variety of animals, including humans. They replicate in the cytoplasm. Their five genes encode structural proteins in the 3′-5′ order: *N* (nucleoprotein), *P* (polymerase), *M* (matrix), *G* (glycoprotein), and *L* (RNA-dependent RNA polymerase) (Johnson *et al*. 2010). The genes are generally conserved, however the *G*, *L*, and *P* genes have undergone adaptive evolution during diversification of the various members of the viral genus. This is particularly manifest in the *G* gene, whose membrane-expressed glycoprotein is externally exposed to the host immune system and is the only RABV molecule known to induce neutralizing antibodies. All human and animal rabies vaccines are based upon rabies virus (RABV)

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 2.1 Rhabdoviruses associated with bats

Bat family	Bat common name	Bat species	Rhabdovirus
Vespertilionidae	Pallid bat	*Antrozous pallidus*	Rabies virus
Phyllostomidae	Brown fruit-eating bat	*Artibeus concolor*	Rabies virus
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	Rabies virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Rabies virus
Vespertilionidae	Western barbastelle	*Barastella barastellus*	EBLV-1
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	Rabies virus
Phyllostomidae	Gray short-tailed bat	*Carollia subrufa*	Rabies virus
Vespertilionidae	Free-tailed bats	*Chaerephon* sp.	Rhabdovirus
Molossidae	Southern dog-faced bat	*Cynomops planirostris*	Rabies virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Rabies virus
Phyllostomidae	White-winged vampire bat	*Diaemus youngi*	Rabies virus
Phyllostomidae	Hairy-legged vampire bat	*Diphylla ecaudata*	Rabies virus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Kumasi rhabdovirus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Lagos bat virus
Pteropodidae	Gambian epauletted fruit bat	*Epomophorus gambianus*	Lagos bat virus
Pteropodidae	Wahlberg's epauletted fruit bat	*Epomorphorus wahlbergi*	Lagos bat virus
Pteropodidae	Dobson's epauletted bat	*Epomops dobsoni*	Lagos bat virus
Vespertilionidae	Argentine brown bat	*Eptesicus furinalis*	Rabies virus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	American bat vesiculovirus
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	*E. isabellinus* rhabdovirus 1
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	*E. isabellinus* rhabdovirus 2
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	*E. isabellinus* rhabdovirus 3
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	*E. isabellinus* rhabdovirus 4
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	*E. isabellinus* rhabdovirus 5
Vespertilionidae	Isabell's serotine	*Eptesicus isabellinus*	EBLV-1
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	EBLV-1
Molossidae	Black-bonneted bat	*Eumops auripendulus*	Rabies virus
Molossidae	Wagner's bonneted bat	*Eumops glaucinus*	Rabies virus
Molossidae	Patagonian dwarf bonneted bat	*Eumops patagonicus*	Rabies virus

Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Rabies virus
Rhinolophidae	Jone's roundleaf bat	*Hipposideros jonesi*	Kolente virus
Rhinolophidae	Striped roundleaf bat	*Hipposideros vittatatus*	Fikirini rhabdovirus
Rhinolophidae	Striped roundleaf bat	*Hipposideros vittatatus*	Shimoni virus
Vespertilionidae	Big-eared brown bat	*Histiotus macrotus*	Rabies virus
Vespertilionidae	Small big-eared brown bat	*Histiotus montanus*	Rabies virus
Vespertilionidae	Savi's pipistrelle	*Hypsugo savii*	*Hypsugo savii* rhabdovirus 1
Vespertilionidae	Savi's pipistrelle	*Hypsugo savii*	EBLV-1
Vespertilionidae	Desert red bat	*Lasiurus blossevillii*	Rabies virus
Vespertilionidae	Hoary bat	*Lasiurus cinereus*	Rabies virus
Vespertilionidae	Southern yellow bat	*Lasiurus ega*	Rabies virus
Vespertilionidae	Northern yellow bat	*Lasiurus intermedius*	Rabies virus
Vespertilionidae	Seminole bat	*Lasiurus seminolus*	Rabies virus
Phyllostomidae	Mexican long-nosed bat	*Leptonycteris nivalis*	Rabies virus
Phyllostomidae	Lesser long-nosed bat	*Leptonycteris yerbabuenae*	Rabies virus
Phyllostomidae	Waterhouse's leaf-nosed bat	*Macrotus waterhousii*	Rabies virus
Phyllostomidae	Little big-eared bat	*Micronycteris megalotis*	Rabies virus
Miniopteridae	African long-fingered bat	*Miniopterus africanus*	Rhabdovirus
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	Lleida bat lyssavirus
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	EBLV-1
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	Duvenhage virus
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	Lleida bat lyssavirus
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	*M. schreibersi* rhabdovirus 1
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	West Caucasian bat virus
Molossidae	Coiban mastiff bat	*Molossus coibensis*	Rabies virus
Molossidae	Thomas's mastiff bat	*Molossus currentium*	Rabies virus
Molossidae	Pallas's mastiff bat	*Molossus molossus*	Rabies virus
Molossidae	Black mastiff bat	*Molossus rufus*	Rabies virus
Molossidae	Sinaloan mastiff bat	*Molossus sinaloae*	Rabies virus

(*Continued*)

TABLE 2.1 (Continued)

Bat family	Bat common name	Bat species	Rhabdovirus
Mormoopidae	Ghost-faced bat	*Mormoops megalophylla*	Rabies virus
Vespertilionidae	Greater tube-nosed bat	*Murina leucogaster*	Irkut virus
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	Aravan virus
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	EBLV-1
Vespertilionidae	Chilean myotis	*Myotis chiloensis*	Rabies virus
Vespertilionidae	Pond bat	*Myotis dasycneme*	EBLV-1
Vespertilionidae	Pond bat	*Myotis dasycneme*	EBLV-2
Vespertilionidae	Daubenton's bat	*Myotis daubentonii*	EBLV-2
Vespertilionidae	Cinnamon myotis	*Myotis fortidens*	Rabies virus
Vespertilionidae	Large mouse-eared bat	*Myotis myotis*	EBLV-1
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Khujand virus
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bokeloh virus
Vespertilionidae	Black bat	*Myotis nigricans*	Rabies virus
Vespertilionidae	Black bat	*Myotis nigricans*	EBLV-1
Vespertilionidae	Cave myotis	*Myotis velifer*	Rabies virus
Vespertilionidae	Yuma myotis	*Myotis yumanensis*	Kern Canyon virus
Noctilionidae	Greater bulldog bat	*Noctilio leporinus*	Rabies virus
Vespertilionidae	Common noctule	*Nyctalus noctula*	EBLV-1
Molossidae	Broad-eared bat	*Nyctinomops laticaudatus*	Rabies virus
Molossidae	New World free-tailed bat	*Nyctinomops macrotis*	Rabies virus
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	Rabies virus
Phyllostomidae	Lesser spear-nosed bat	*Phyllostomus elongates*	Rabies virus
Phyllostomidae	Great spear-nosed bat	*Phyllostomus hastatus*	Rabies virus
Vespertilionidae	Kuhl's pipestrelle	*Pipistrelles kuhlii*	EBLV-1
Vespertilionidae	Nathusius's pipistrelle	*Pipistrelles nathusii*	EBLV-1
Vespertilionidae	Nathusius's pipistrelle	*Pipistrelles nathusii*	EBLV-2
Vespertilionidae	Common pipistrelle	*Pipistrelles pipistrelles*	EBLV-1
Phyllostomidae	White-lined broad-tailed bat	*Platyrrhinus lineatus*	Rabies virus rhabdovirus 1

Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	EBLV-1
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	*Plecotus auritus*
Vespertilionidae	Gray big-eared bat	*Plecotus austiacus*	EBLV-1
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	Rabies virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnellii*	Rabies virus
Mormoopidae	Wagner's mustached bat	*Pteronotus personatus*	Rabies virus
Pteropodidae	Black flying fox	*Pteropus alecto*	Australia bat lyssavirus
Pteropodidae	Lyle's flying fox	*Pteropus lylei*	Lyssavirus
Pteropodidae	Indian flying fox	*Pteropus medius* (*P. giganteus*)	Gannoruwa bat lyssavirus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalis*	Australia bat lyssavirus
Pteropodidae	Little red flying fox	*Pteropus scapulatus*	Australia bat lyssavirus
Pteropodidae	Spectacled flying fox	*Pteropus spiciliatus*	Australia bat lyssavirus
Vespertilionidae	Little yellow bat	*Rhogeessa parvula*	Rabies virus
Vespertilionidae	Black-winged little yellow bat	*Rhogeessa tumida*	Rabies virus
Rhinolophidae	Little Japanese horseshoe bat	*Rhinolophus cornutus*	Oita virus
Rhinolophidae	Great horseshoe bat	*Rhinolophus ferrumequim*	EBLV-1
Rhinolophidae	Great horseshoe bat	*Rhinolophus ferrumequim*	*R. ferrumequim* rhabdovirus 1
Rhinolophidae	Eloquent horseshoe bat	*Rhinolophus hildebrandtii eloquens*	Mount Elgon bat virus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Lagos bat virus
Emballonuroidea	Yellow-bellied sheath-tailed bat	*Saccolaimus flaviventris*	Australia bat lyssavirus
Vespertilionidae	Asiatic lesser yellow house bat	*Scotophilus kuhii*	Lyssavirus
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	Rabies virus
Molossidae	Wrinkle-lipped free-tailed bat	*Tadarida plicata*	Lyssavirus
Molossidae	European free-tailed bat	*Tadarida teniotis*	EBLV-1
Molossidae	Free-tailed bats	*Tadarida* sp.	Gossas virus
Emballonuroidea	Theobald's tomb bat	*Taphozous theobaldi*	Lyssavirus
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	Rabies virus
Vespertilionidae	Parti-colored bat	*Vesperilio murinus*	EBLV-1

or its derivatives. Relatedness of the various lyssaviruses to the external portion of RABV *G* gene DNA sequences may aid in predicting the efficacy of the vaccines to newly discovered lyssaviruses (McElhinney *et al*. 2011). This is particularly important since the RABV vaccine does not protect against all of the currently known lyssaviruses. G recognizes host cells and is thus important to lyssavirus tropism as well as virulence (Calisher & Ellison 2012; Voloch *et al*. 2014). The *L* gene may have been the primary target of adaptive evolution early during lyssavirus diversification (Voloch *et al*. 2014).

Evolutionary analysis suggests that all lyssaviruses likely originated in bats and afterwards underwent adaptation. In addition to clinically rabid bats, healthy bat populations may serve as viral reservoirs as evidenced by detection of European bat lyssavirus (EBLV)-1 RNA in oropharyngeal cavities of *Eptesicus isabellinus* (Vazquarez-Morón *et al*. 2008). The *Lyssavirus* genus currently contains thirteen lyssaviruses and two tentative species. All of these, with the exception of Mokola virus and Ikoma lyssavirus, have been detected in bats, which serve as reservoir hosts. Carnivores however, often play major or critical roles in transmission to humans (Calisher & Ellison 2012; Schatz *et al*. 2014b). RABV prevalence in large gregarious bat colonies is usually less than 1% but 70% of the bats may produce anti-viral antibodies (from Dzikwi *et al*. 2010), suggesting that bats might be exposed often, allowing them to develop protective immunity in the absence of infection.

EBLV-1, EBLV-2, Bokeloh virus, and Lleida bat lyssavirus have been detected in bats in Europe; West Caucasian bat lyssavirus, Irkut virus, Aravan virus, and Khujand virus in Eurasia; Lagos bat virus (LBV), Duvenhage virus (DUVV), Shimoni virus, and West Caucasian bat lyssavirus in Africa; Australian bat lyssavirus (ABLV) in Australia; and RABV in the Americas (Reynes *et al*. 2004; Schatz *et al*. 2014b). RABV causes most human rabies cases worldwide and may be divided into two lineages: one transmitted primarily by carnivores with worldwide distribution and the other transmitted by bats and present only in the Americas (Reynes *et al*. 2004). In addition to RABV, DUVV, EBLV-1 and -2, ABLV, Irkut virus, and Mokola virus cause fatal disease in humans (Reynes *et al*. 2004).

Three phylogroups of lyssavirus are recognized. Phylogroup I has the most species diversity and is composed of RABV, EBLV-1, EBLV-2, Bokeloh virus, DUVV, Irkut virus, Aravan virus, Khujand virus, and ABLV. Phylogroup II is composed of two African species: LBV and Mokola virus. Phylogroup III contains Shimoni bat virus, West Caucasian bat lyssavirus, and Ikoma lyssavirus, the latter two of which, based on sequence homology, may be sister viruses. Lleida bat lyssavirus has also been recently placed in this phylogroup (Voloch *et al*. 2014; Weir *et al*. 2014a).

2.2.1 Rabies virus

In the majority of the world, domestic animals (dogs and cats) and wildlife are the reservoirs and vectors of RABV. Bat transmission of RABV to humans occurs only in the Americas and involves hematophagous, frugivorous, and insectivorous bat groups, in addition to transmission to humans by other animals. In the US, brain smears of 92% of grounded *Tadarida brasiliensis* ($n = 321$) bore rabies antigens (Davis *et al*. 2012). In Latin America, two epidemiological forms of rabies exist: (1) urban, in which dogs are the primary reservoir host; and (2) an independent enzootic sylvatic form transmitted by bats and wild carnivores (Condori-Condori *et al*. 2013). While numbers of human RABV

infections in the urban cycle has decreased in the area due to vaccination of domestic dogs, sylvatic bat-associated case numbers in cattle and humans has increased significantly in some countries during the past decade despite a stable number of cases in bats (Condori-Condori *et al*. 2013; Escobar *et al*. 2015; Moran *et al*. 2015). The reported increase in human cases may reflect an actual rise in numbers of human infections or improved detection and reporting as a result of greater awareness of the disease by health care professionals and public health workers. Increased interactions may also be occurring between vampire bats and humans due to loss of their other prey, ecological disturbances, evolutionary changes in bat populations, or increased surveillance. Another factor to be considered is the growth in number of cattle ranches, which provide abundant food sources within a small area. This readily available food source together with changes in habitat contribute to vampire bats becoming dependent upon agricultural animals for food, increasing in number, and increasing the number of human exposures.

Escobar *et al*. (2015) report 333 bat species as being present in 24 Latin American or Caribbean countries. Of these, 75 (22.5%) have been confirmed as being occasionally rabies-positive, with the greatest number of rabid bat species found in Brazil (43), Mexico (31), and Argentina (13). It should be noted, however, that these numbers may reflect better surveillance in these countries. See Escobar *et al*. (2015) for the complete list. It should be noted that although Brazil also has the second greatest number of bat species in the region (155 species), the number of rabies-positive species does not correlate with the number of total bat species present in a given country. Rabies-positivity varied by family: 64% of Vespertilionidae (25 species), 50% of Noctilionidae (1 species), 44% of Mormoopidae (4 species), 17% of Phyllostomidae (29 species), and 5% of Emballonuridae (1 species). By diet, 100% of hematophagous bats (3 species) were reported to be rabies-positive, 60% of carnivorous bats (3 species), 27% of insectivorous (50 species), 19% of nectivorous (5 species), 13% of frugivorous (10 species), and 11% of omnivorous bats (4 species). In terms of antigenic variation, Brazil had the greatest number of RABV variants (9), followed by Mexico (7), and Argentina (6). All other countries had 4 or less RABV variants. While most of the rabies-positive bat species are listed as of Least Concern, one species is currently endangered (*Leptonycteris nivalis*) and three have declining numbers (*Leptonycteris yerbabuenae, Eumops perotis*, and *Mormoops megalophylla*). Attempts to limit human contact with potentially rabid bats have, however, have led to indiscriminate killing of bats by poisoning or burning and dynamiting their roosts. Very large numbers of economically and ecologically important bats are being killed, posing a major problem for bat conservation but also to impoverished human populations that rely on bat pollination of crops and also on guano.

Throughout its range from Mexico to Argentina, the primary reservoir for RABV is currently *Desmodous rotundus* (the common vampire bat) which preys on livestock (cattle, horses, and pigs), but may also feed upon other animals, including humans (Condori-Condori *et al*. 2013; Moran *et al*. 2015). Between 2004 and 2005, 98 human cases resulted from contact with vampire bats (Johnson *et al*. 2010). The first indication of a link between vampire bats and fatal neurological disease was in Brazil in 1911, later confirmed in 1921 (Johnson *et al*. 2010). Vampire bat numbers are believed to have increased after European colonization due to increased host availability in the form of cattle and horses (Johnson *et al*. 2010). Vampire bat-associated rabies was first reported in humans during the 1930s in Trinidad (reviewed by Moran *et al*. 2015).

Forty-one of the 178 species of Brazilian bats have been found to be infected by RABV to a greater or lesser extent (Casagrande *et al*. 2014). The incidence of infection in these species may not accurately reflect the true incidence of infection under normal conditions, since most of the tested bats were either dead or ill, thus introducing sampling bias. Recent data from Brazil found that 64.8% of infected bats were from non-hematophagous species, 17.6% were hematophagous species, and remainder were not identified (Casagrande *et al*. 2014). When brain tissue from urban Brazilian bats was assayed, no infection was seen in hematophageous bats, despite finding that 7.2% of these animals had neutralizing antibodies. By contrast, 2% of non-hematophageous bats' brains were infected. While almost 90% of the infected bats were insectivores of the Molossidae family, these bats are more likely to be in contact with humans than the vampire bats, which tend to not be found in human dwellings, and also may reflect sampling bias. Over 40% of these were *Molossus* species, primarily *Molossus rufus*; almost 40% were *Eptesicus* species, predominantly *Eptesicus furinalis*, and 17% were *Myotis nigricans*. Only about 10% of the RABV-positive bats were frugivorous (*Artibeus lituratus*) (Casagrande *et al*. 2014).

In RABV-positive animals, RNA was detected in the brain and salivary glands of 100% of the animals. Other sites of infection were for frugivorous and insectivorous bats, respectively: tongue (92% and 85%); brown fat (82% and 77%), lung (62% and 77%), heart (42% and 77%), stomach (92% and 64%), liver (38% and 67%), spleen (43% and 27%), bladder (73% and 88%), kidney (77% and 38%), intestine (77% and 38%), and feces (38% and 42%), localizing in similar body components regardless of bat species (Allendorf *et al*. 2012).

In a phylogenetic study of the complete *N* gene, Peruvian bat-rabies can be divided into two major groups: one associated with the common vampire bat, with lineages I–IV in Peru and the second group associated with insectivorous bats, subdivided into three lineages (Condori-Condori *et al*. 2013). Linage I RABV is wide-spread in Peru and northern South America; lineage II, in Central Peru, Brazil, and Uruguay; and lineage III, in a restricted part of the central Peruvian Amazon. Lineage IV was the most frequently isolated lineage and is very prevalent in cattle and vampire bats in the inter-Andean valleys, the rainforests of northern Peru, and northern Columbia (Condori-Condori *et al*. 2013). Vampire bats are not migratory and typically inhabit locations with an altitude of under 1800 m. The inter-Andean valleys inhabited by lineage IV RABV, however, have an average altitude greater than 2000 m. Since they are a major region used by cattle ranchers, the bats in this area may have adapted in order to more optimally utilize this prey (Cordori-Condori *et al*. 2013). Cordori *et al*. (2013) hypothesized that since more than one viral lineage may coexist in a given geographical area, the different lineages might be maintained in distinct bat metapopulations.

Due to the success in control of dog-associated rabies in Brazil, transmission by bats grew to 70% of the human rabies cases between 2004 and 2013, with dogs only responsible for 22% of human infections (Casagrande *et al*. 2014). A similar situation occurred in Peru from 2002 to 2007 (Condori-Condori *et al*. 2013). In Latin America between 2004 and 2013, of the 243 cases of bat-associated human rabies cases, 91.4% were caused by hematophagous bats. It appears, therefore, that while the majority of infected bats are non-hematophagous, transmission to humans occurs by far more often from hematophagous species, suggesting that rabies control effects might be most effective if concentrated on the vampire bats in Latin America. Culling of vampire bats is the

primary means of controlling bat-associated rabies, although other means are also being employed (see Section 2.2.6). Culling, however, has not decreased the numbers of infected livestock (Johnson *et al*. 2010; Condori-Condori *et al*. 2013).

In the US, where one to two cases of human rabies are reported each year, a study of over 24 000 bats found that, of suspected animals, 5.9% were RABV-positive (McElhinney *et al*. 2011; Cordori-Condori *et al*. 2013). Both insectivorous and frugivorous species may transmit disease to humans and 77% of all indigenous bat species in the US and Canada contain members that may be occasionally infected (Johnson *et al*. 2010) with the number of cases increasing. After dog-vaccination control programs were instituted in North America, the number of bat-associated human cases of rabies outnumbered those from dogs. From 1950 to 2007, 61 human rabies cases were bat variants, obtained transmitted primarily by bites, but 5 cases were linked to organ transplantation (Johnson *et al*. 2010). Bat RABV variants are also found in skunks and gray foxes in North America (Escobar *et al*. 2015).

2.2.1.1 Rabies – the Disease Rabies was first described in detail in 1530 by Girolamo Fracastoro of Verona, who also described the routes of transmission. The disease and its connection with dogs have been known far longer, however, being mentioned in the Eshuma Code of Babylon in the 23rd century BCE (Calisher & Ellison 2012). In Sanskrit, "rabbas" means to do violence, while in Latin, "rabere" refers to raving and madness. The genus name is derived from Greek mythology. The goddess Lyssa, one of the Maniae, was believed to have turned Actaeon into a stag and driven his dogs mad in order to kill the youth for gazing upon Artemis as she bathed (Calisher & Ellison 2012).

Rabies is an acute, progressive encephalitis (inflammation of brain tissue) with a fatality rate greater than 99.9%. The disease kills approximately 55 000 people each year, especially in the developing nations of Africa and Asia (reviewed by He 2014a). Infection with several other lyssaviruses also causes disease that is fatal in almost all incidences in the absence of post-exposure prophylaxis given prior to disease onset. The incubation period ranges from several days to several years, but typically is 3–8 weeks. Symptoms of disease may include apprehension and confusion, paresthesia at the site of exposure, pharyngitis, malaise, headache, and fever, followed by muscle aches, flaccidity of the limbs, and vomiting. Fear of water (hydrophobia) is common and gives the disease its common name. Aerophobia is also common as are spasms of the swallowing muscles and excessive salivation. In "furious rabies," later symptoms include aggression, delirium, and convulsions. The remaining third of cases develop paralysis of limbs and respiratory muscles with continued consciousness, but often in the absence of phobic convulsions. Coma and death, often due to cardiac failure, occur within 1–2 weeks (Johnson *et al*. 2010). RABV-infected neurons contain Negi-bodies, cytoplasmic inclusions that have been suggested to consist of viral particles in an amorphous matrix, in 70.9% of infected humans (Jackson *et al*. 2001). Histological changes in non-RABV rabies include more inflammation with perivascular cuffing than is caused by RABV, infiltration into and necrosis of parts of the brainstem and hippocampus, and inclusion bodies that are not Negri bodies (Johnson *et al*. 2010).

Humans were helpless against this essentially universally fatal disease until 1885, when Louis Pasteur developed a "therapeutic" vaccine (post-exposure prophylaxis or PEP) that could prevent rabies if given early after the bite of a rabid dog. The vaccine

consisted of attenuated (weakened, nonpathogenic), live RABV. After using his attenuated vaccine to successfully prevent rabies in 50 experimentally infected dogs, Pasteur was able to prevent disease in a 9-year-old boy, Joseph Meister, who had received multiple bites from a rabid dog (reviewed by Smith 2012). With this advance, the course of rabies in humans was altered from death to life.

Although lyssaviruses have been reported to be the only group of viruses known to cause disease in bats and only in small numbers following experimental infection (reviewed by He 2014a), other viruses also cause severe infection in bats, especially pregnant or otherwise immunocompromised bats. These viral groups or viruses include the following: rubulaviruses. Belinga bat virus, Eptesipox virus, adenoviruses, gamma-herpesviruses, Lloviu virus, and Jeilongvirus.

2.2.1.2 Rabies in terrestrial carnivores There have been questions concerning the relative importance of rabies transmission by bats or other wildlife in some developing regions of the world, such as South Africa, where dogs are the primary reservoir host. In developed areas, wild carnivorous are the primary reservoirs (Heymann 2008). In a 12-year study of 2697 travelers from 24 countries of six continents receiving PEP for rabies following animal exposure, the most commonly rabies-associated animals were dogs (60%), nonhuman primates (24%), cats (10%), and bats (2%) (Gautret *et al*. 2015). Those human exposures resulting from contact with nonhuman primates or cats were most likely to occur in southeastern South-Central Asia. The countries with the most bat-associated exposures were Indonesia, French Guiana, Peru, Mexico, and Suriname. These exposures were most common during July–September. Almost all travelers reporting bat exposure were aged 15–44 years. Since this study focused only on travelers receiving PEP, it may not reflect exposures experienced by area residents, but may still give us a glimpse into the relative risk of contracting rabies directly from bats in comparison with other animals.

Rabies may infect any mammal but, in addition to bats, has been more commonly associated with dogs, wolves, foxes, coyotes, jackals, raccoon dogs, cats, raccoons, skunks, shrews, and mongooses. Infection of rats, mice, chipmunks, squirrels, rabbits, and opossum are much less frequent (Heyman 2008).

2.2.2 Other lyssaviruses of bats

2.2.2.1 Other lyssaviruses of bats in Europe Europe is home to several lyssaviruses, EBLV-1 and EBLV-2, which are linked to fatal human bat-borne rabies. Bokeloh virus and Lleida bat lyssavirus have also been detected in European bats. EBLV-1 is by far the most common of the European lyssaviruses in animals and is found primarily in Germany (particularly in lower elevations in the northern part of the country), Denmark, the Netherlands, and Poland (McElhinney *et al*. 2011). It is divided into two lineages: the highly conserved EBLV-1a, which has an east–west distribution and is found in France, the Netherlands, Denmark, Germany, Poland, Ukraine, and Russia; and the more genetically diverse EBLV-1b, distributed in a north–south manner with four sub-lineages in Spain, France, southern Germany, Poland, and the Netherlands (Picard-Meyer *et al*. 2014; Schatz *et al*. 2014a). The mutation rate of EBLV-1 is one of the lowest reported for RNA viruses. It is postulated to have arisen 500–750 years ago,

suggesting strong evolutionary stability with its host. EBLV-1 is found in bats throughout mainland Europe, but active viral infection has not been reported in the UK. The less common EBLV-2, by contrast, has been found only in the UK and the northern European countries of the Netherlands, Germany, and Switzerland (Johnson *et al*. 2010; Calisher & Ellison 2012). The difference in the presence or absence of these viruses in the UK may be due to the greater propensity of EBLV-2's primary bat host to migrate between the UK and the mainland than that of EBLV-1's bat host, despite the presence of the latter bat throughout much of Southern England (Johnson *et al*. 2010). EBLV-2 is also less virulent than EBLV-1 in animal models. Both of these European viruses are more closely related to Duvenhage virus than to RABV and are less pathogenic for humans than RABV (Calisher & Ellison 2012).

Two human cases each of EBLV-1 and EBLV-2 have been reported in Europe (Ukraine and Russia; Finland and the UK, respectively) (Johnson *et al*. 2010). Additionally, there have been very rare spill-over infections of EBLV-1 to 2 French domestic cats, 2 Danish sheep, and a German stone marten (Schatz *et al*. 2014b).

EBLV-1 has been found in some European bats, particularly *E. serotinus*. *E. serotinus* is widespread in Europe. The other principal bat hosts for European lyssaviruses are *E. isabellinus, Myotis daubentoniii, Myotis dasycneme*, and *Miniopterus schreibersi*. Notably, 11 of 21 tested species of European bats were positive for lyssavirus neutralizing antibodies, of which four had no detectable viral antigen (*Barastella barastellus, Myotis blythii, Myotis myotis, Rhinolophus ferrumequim*) (Schatz *et al*. 2012). First found in a bat in Germany in 1954, in the 30 years between 1977 and 2012, 1039 European bat rabies virus infections were reported, primarily EBLV-1 but also 24 of EBLV-2. Besides Germany, EBLV-1 in bats has been found in Denmark, the Netherlands, Poland, France, Spain, and Yugoslavia (Johnson *et al*. 2010). The widely distributed *E. serotinus* serves as the primary bat vector of EBLV-1, with greater than 95% of them being in this bat species (McElhiney *et al*. 2011). One lineage of EBLV-1, however, is restricted to south of the Iberian Peninsula, where *E. isabellinus* serves as the primary reservoir species (McElhinney *et al*. 2011). A study in southern Spain detected anti-EBLV-1 antibodies and RNA in 2.8% of oropharyngeal swabs of healthy *E. isabellinus* ($n = 1226$), indicative of subclinical infection. Infected colonies also showed different temporal patterns of circulation, suggesting independent endemic circulation in this region (Vázquarez-Morón *et al*. 2008). Interestingly, the same study found a negative correlation between the presence of viral RNA in the brain and body condition. *E. isabellinus* itself is restricted to Southern Spain and Northern Africa. Of note, no virus has been isolated from the North African *E. isabellinus* population, despite evidence for genetic flow between these bats and those in Spain (McElhinney *et al*. 2011). EBLV-1 RNA has also been detected in several *Pipestrelles pipistrelles*, a *Pipstrelles nathusii, Pipstrelles auritus, Pipistrelles austiacus*, and *Tadarida teniotis* in Germany and Spain (Schatz *et al*. 2014a). Three cases of EBLV-1 were also reported in *Nyctalus noctula* in Yugoslavia and one case in *Vesperilio murinus* in Russia (Picard-Meyer *et al*. 2014).

Two *Myotis* species host EBLV-2, with the majority of virus isolations coming from the widely distributed *M. daubentoni* and, due to its more restricted range, EBLV-2 in *M. dasycneme* found in the Netherlands and Central and Eastern Europe (Johnson *et al*. 2010; McElhinney *et al*. 2011). EBLV-2 RNA has also been found in oropharyngeal swabs from several healthy *M. daubentoni*. In a 2012 study of Swiss bats ($n = 237$), only three neutralizing antibody-positive *M. daubentoni* were found and, despite the

detection of EBLV-2 RNA from swabs of one of these bats, no infectious virus was found (Megali *et al*. 2010). EBLV-2 is also endemic at a low level in dead members of these two *Myotis* species in the UK (England and Scotland) (Banyard *et al*. 2009).

Only three isolations of Bokeloh virus have been reported, all from *M. nattereri*, despite the abundance of this bat species in Europe. Bokeloh virus was isolated twice in German bats (2010 and 2012) and once from a French bat in 2012 (Freuling *et al*. 2013). The three isolates are most related to Khujand virus and EBLV-2, with 80% and 79% genetic similarity, respectively (Nolden *et al*. 2014). Bokeloh virus antigen was detected in a rabid bat's brain, but not the salivary glands (McElhinney *et al*. 2011). Mice, especially young mice, are susceptible to experimental Bokeloh virus infection by the intracerebral or intramuscular route. High levels of species-specific neutralizing antibody are protective (Nolden *et al*. 2014).

Between 1977 and 2011, a yearly average of 34 EBLV-infected bats was reported (McElhinney *et al*. 2011). It should be noted that several studies have found significant variations in yearly incidence of bat EBLV-1. Picard-Meyer *et al*. (2014) reported that infections were most commonly reported in the autumn in France (~34% of the infections were noted in autumn as compared with 12–15% in each of the three other seasons), while other reports found a spring and autumn seasonality or an increase in seropositivity in the summer in some Spanish bats. Seasonal variations in various bat life cycles (times of migration, mating, hibernating, pregnancy, lactation, formation of maturity colonies) could account for some of the reported differences in virus prevalence and transmission between bats (Schatz *et al*. 2012; Picard-Meyer *et al*. 2014). Bats may have some natural protection against EBLV-associated disease since a 12-year surveillance study of two Spanish *M. myotis* colonies found a high level of immunity to EBLV-1 after its rapid dissemination through bat colonies in the absence of significantly increased mortality (Amengual *et al*. 2007).

In the Iberian Peninsula in 2012, Lleida bat lyssavirus was detected by molecular means, but not isolated, from a single *Miniopterus schreibersi* (Schatz *et al*. 2014b).

2.2.2.2 Other lyssaviruses of bats in Africa Duvenhage virus caused three cases of human rabies in adults in 1971, 2006, and 2007. Two of these were from South Africa and the other was from Kenya. It is transmitted by bites or scratches of unidentified species of bat(s), at least one of which was *Miniopterus schreibersii*.

The Lagos bat virus is more wide-spread in Africa and has been found in several species of bats, *Eidolon helvum, Epomops dobsoni, Epomophorus gambianus, Epomophorus wahlbergi*, and *Rousettus aegyptiacus*, as well as cats, dogs, and mongooses (Johnson *et al*. 2010; Calisher & Ellison 2012). In a study of 350 Nigerian bats, no lyssavirus antigen or virus (RABV, DUVV, West Caucasian bat virus, LBV, and Mokola virus) was isolated from the brains, however, neutralizing antibodies against LBV were detected in 19% of 140 bats' serum (primarily *E. helvum* and the remainder, *E. gambianus*) (Dzikwi *et al*. 2010). In a study from South Africa, 10–15% of a group of several hundred dying or dead bats carried Lagos bat virus (Johnson *et al*. 2010). LBV can cause fatal infection in cats, even if vaccinated against RABV. Lagos bat virus has not been reported to cause infection in humans nor has Shimoni virus, its close relative, isolated from *Hipposideros vittatatus* (initially identified as *Hipposideros commersoni*) in Kenya (Calisher & Ellison 2012).

Mokola virus was first found in Nigerian shrews in 1968 and was then isolated in 1971 from two young Nigerian children with central nervous system illness. One of the

children recovered fully after a clinical course consisting of fever, pharyngitis, and convulsions, while the other child died (Johnson *et al*. 2010). In addition to shrews, Mokala virus has been isolated from an unidentified rodent species and several dogs, but cats, even those vaccinated against RABV, are by far the most common host. Besides Nigeria, the virus has been reported in Cameroon, the Central African Republic, Ethiopia, Zimbabwe, and South Africa (Johnson *et al*. 2010). Mokola virus and Ikoma virus have not yet been associated with bats (Kgaldi *et al*. 2013). The latter was isolated from a rabid civit (Voloch *et al*. 2014).

2.2.2.3 Other lyssaviruses of bats in Asia

2.2.2.3.1 Other lyssaviruses of bats in Western and Central Asia In the Caucasus region, several lyssaviruses have been detected in bats: Aravan virus in *Myotis blythii* in Kyrgistan, Khujand virus in *Myotis mystacinus* in Kyrgistan, Irkut virus in *Murina leucogaster* in Eastern Siberia, and West Caucasian bat viruses in *M. schreibersi* in the West Caucasus Mountains (Reynes *et al*. 2004; Calisher & Ellison 2012). Aravan virus is most closely related to Duvenhage virus and EBLV-1, while Khujand virus is most closely related to EBLV-2. One fatal human infection with Irkut virus has been reported (Calisher & Ellison 2012). In addition to its isolation from *Miniopterus schrebersii* near the Georgian–Turkish border, West Caucasian bat virus has been identified in Kenya (McElhinney *et al*. 2011; Voloch *et al*. 2014). It is the most genetically divergent of the lyssaviruses (McElhinney *et al*. 2011).

2.2.2.3.2 Other lyssaviruses of bats in Northern and Southeastern Asia Rabies is endemic to Cambodia and human infection has yet to be linked to bats. The majority of cases are transmitted by dogs (Reynes *et al*. 2004), even though ELISA and the rapid fluorescent focus inhibition test detected lyssavirus antigen in 31% of frugivorous bats (10/32 samples from 5 bat species) and 18% of insectivorous bats (20/114 samples from 11 bat species). EBLV-1 and ABLV antigens are detected most frequently (10% and 9%, respectively). Of the three species of frugivorous bats which were antibody-positive, *Pteropus lylei* had the highest rate of exposure (11%; $n = 228$). Of the five species of insectivorous bats with viral antigen, *Tadarida plicata* had the highest rate of infection (27%), followed by *Scotophilus kuhlii* (19%), and *Taphozous theobaldi* (16%). However, no antigen was found by IFA in the brains of over 1000 Cambodian bats and no infectious virus was found in the brains of 24 bats (Reynes *et al*. 2004). A 2016 study (Gunawardena *et al*. 2016) isolated a novel lyssavirus, Gannoruwa bat lyssavirus, from grounded Indian flying foxes (*Pteropsus medius*, formerly known as *P. giganteus*) in Sri Lanka ($n = 62$). Almost all of these bats were dead or died shortly after capture. Negri bodies were also present in the bats' brains.

2.2.2.4 Other lyssaviruses of bats in Australia and Oceania

While RABV is absent from Australia, a rabies-like virus that is fatal to humans is present, the Australian bat lyssavirus. All three human cases reported to date developed the encephalitic form of ABLV rabies prior to death (Francis *et al*. 2014). This lyssavirus was first found in brain tissue of the frugivorous bat *Pteropus alecto* in 1996. It has since been divided into two distinct lineages: ABLVp in frugivorous bats; and ABLVs in the insectivorous microchiroptera. All four of the frugivorous flying fox species in Australia (*P. alecto, Pteropus poliocephalis, Pteropus scapulatus*, and *Pteropus spiciliatus*) can be infected

but only a single species of insectivorous microchiroptera, *Saccolaimus flaviventris* is known to be infected (Calisher & Ellison 2012; Francis *et al*. 2014). Although ABLV has not yet been isolated from any of the other 64 microchiroptera in Australia, ABLV-specific antibodies have been found in five species of flying foxes, suggesting their exposure to, and perhaps infection with, ABLV (Weir *et al*. 2014a). It should be noted that despite the fact that three of the above species of *Pteropus* are also found in Papua New Guinea and two species are found in parts of Indonesia, ABLV has not been isolated outside of Australia, even though neutralizing antibodies to ABLV have been detected in six species of Philippian bats (Weir *et al*. 2014a). While present in less than 1% of healthy, wild bats, ABLV is detected in 5–10% of ill, injured, and orphaned *Pteropus* in Australia, with the percentage highly variable among species, ranging from 1% in dysfunctional *P. spiciliatus* to 17% in *P. scapulatus* and up to 63% in ill *S. flaviventris* (Weir *et al*. 2014a).

ABLV has thus far been transmitted to humans through contact with saliva, with no virus being detected in bat blood, urine, or feces (Francis *et al*. 2014). ABLV transmission to two horses has also been reported, but the virus has not been detected in dogs, even after contact with infected bats. Experimental inoculation of ABLV into either dogs or cats resulted in seroconversion, but only minor behavioral changes without overt clinical disease or viral replication over a 3-month time period. Significantly, one of the infected humans had an incubation period of 27 months, suggesting that a longer period of study is necessary to truly assess disease potential in other mammals (Weir *et al*. 2014a). ABLV is able to infect not only human and horse cell lines, but also small rodent, rabbit, and cat embryonic cell lines, indicating expression of its cellular receptor in a wide variety of mammals and suggesting the possibility of a broader host range than has been so far reported (Weir *et al*. 2014a). It should be noted that experimental infection of cell lines does not necessarily mean that the species from whom the original cells were collected are themselves able to be infected, especially in the presence of an immune response in a potential animal host.

2.2.3 Lyssavirus transmission

Infection is typically linked to exposure to saliva from infected animals, particularly via bites or scratches. The only two instances in which this route was suspected to have occurred between humans were later disproven. Infection due to laboratory exposure to saliva and nervous tissue from animals or humans has been found, however (Francis *et al*. 2014). Transmission via transplanted infected corneas, organs, or blood vessels has been documented. Air-borne infection in caves with high numbers of infected bats has also been rarely seen (Heymann 2008) and may not occur in healthy animals. Infection of cattle by the bite of vampire bats is fairly common in Latin America. Dogs and cats are infectious 3–7 days prior to disease onset while some bats may shed virus as long as 12 days prior to becoming symptomatic (Heymann 2008).

Lyssavirus transmission among bats occurs via saliva during bites, licks, and scratches. It is possible that bat-to-bat transmission may occur by contact with aerosolized saliva or milk or transplacentally (Schatz *et al*. 2014b). Mice have been experimentally infected by the aerosol route by RABV, but not by EBLV-2 (Johnson *et al*. 2006). Once the bat is infected, lyssaviruses are believed to spread in a centrifugal fashion from the brain to the periphery.

Some bats inhabit multispecies colonies for at least part of their life cycle. In one such colony, housing nine different species, including *E. serotinus*, six species developed neutralizing antibodies to EBLV-1, indicating intimate exposure or infection with the virus at some time and interspecies transmission of at least this lyssavirus (Lopez-R 2014). In addition to *E. serotinus*, seropositive bats belonged to the following species: *Hypsugo savii* (highest rate of seropositivity), *P. pipistrelles, P. kuhlii, P. austiacus*, and *Tadarida teniotis*. Several individual *P. austiacus* and *M. myotis* which were recaptured at least once and analyzed over the next 1–8 years, demonstrating that at least bats of these species are able to survive for years after seroconversion (Amengual *et al*. 2007; López-Roig *et al*. 2014), although this does not necessarily mean that infection of these bats had occurred. The overall rate of seroprevalance in this colony was similar in males and females, although there were considerable gender differences in some of the species (López-Roig *et al*. 2014). No gender differences in rates of seropositivity were found in two colonies of *M. myotis*; even though the average overall rate was 36.2% in this gregarious bat species (Amengual *et al*. 2007).

2.2.4 Lyssavirus sites of infection

Schatz *et al*. (2014b) tested naturally infected bats detected by passive rabies surveillance in addition to retrospective studies in Germany. They employed the rabies tissue culture inoculation test (RTCIT), quantitative real-time PCR (RT-qPCR), and immunohistochemical techniques to detect lyssaviruses or their RNA in a variety of tissues and organs. Of 57 *E. serotinus*, EBLV-1 RNA was detected by RT-qPCR in parts of the cerebrum, optic nerves, and autonomic ganglia of the nervous system (100% of bats); parotid and mandibular salivary glands (85%); epithelial cells of the tongue (65%); spleen and bladder (56%); and in the pectoral muscle, heart, and lungs (48%). While viable virus was isolated from many *E. serotinus*' brains, salivary glands, or tongues, and less often from the heart, pectoral muscle, and bladder, RTCIT failed to isolate virus from the lungs, liver, spleen, or kidneys (Schatz *et al*. 2014b). In a separate study, however, EBLV-1 was isolated from lung epithelia of an infected bat in France, suggesting that lungs might, in some cases, play a role in viral excretion (Bourhy *et al*. 1992). The highest viral loads were present in the brain and salivary glands, followed by tongues and kidneys. No viral antigen was found in any tissue from a late-stage fetus of an infected bat (Schatz *et al*. 2014b). In the one *P. nathusii* tested, EBLV-1 was detected in the brain, tongue, and heart. In two *M. daubentonii*, EBLV-2 RNA was detected in the brain and salivary glands of both animals and in the heart, tongue, kidney, and pectoral muscle of one animal. Viable EBLV-2 was also isolated from both bats' brains and salivary glands, the only tested materials (Schatz *et al*. 2014b). Lagos bat virus was also found in epithelium and papillae of the tongues of frugivorous African bats (Markotter 2007). Evidence suggests that the common vampire bats' tongue may be the major site of RABV shedding in the Americas (Viera *et al*. 2011). A large amount of viral antigen is also present in tongues of RABV-infected humans (Jackson *et al*. 1999).

In the nervous system, Purkinje cells and the periaqueductal gray neurons had the greatest proportion of volume consisting of Negri bodies and RABV antigens. Even though all cells containing Negri bodies also stained for RABV antigen, some of the cells with large levels of viral antigen lacked Negri bodies (Jackson *et al*. 2001). In 49 infected individual bats, these inclusions were most commonly

present in Ammon's horn of the hippocampus, medulla, pontine nuclei, spinal cord, and pyramidal cells of the cerebral cortex.

2.2.5 Lyssavirus entry into cells

Rhabdoviruses, in general, enter host cells via receptor-mediated endocytosis. The surface glycoprotein is the responsible viral component. Proposed host cell receptors for RABV include the nicotinic acetylcholine receptor, the neuronal cell adhesion molecule, the p75 neurotrophin receptor, and gangliosides (Weir *et al*. 2014a). None of these has been definitively proven to be necessary and sufficient for lyssavirus glycoprotein binding and cell entry, so it is possible that, similar to HIV, one or more alternative co-receptors may also be required for target cell infection. Work by Weir *et al*. (2013) suggests that ABLVp and ABLVs may indeed utilize different co-receptors. While the cellular receptor for both ABLV is unknown, it appears to be enriched in lipid rafts (Weir *et al*. 2013).

ABLV may be internalized via the clathrin-mediated pathway in human embryonic kidney cells (HEK293) in a process that relies on actin polymerization (Weir *et al*. 2014b). HEK293 cells express several genes that are typically restricted to developing neurons or neural stem cells, thus mimicking the host target cell. After internalization of clathrin-coated pits and fusion with endosomes, the mildly acidic environment in the early endosomes stimulates a conformational change in the viral glycoprotein that in turn allows fusion of the glycoprotein with the vesicle membrane. This is followed by the viral genome's release into the host cell's cytoplasm. RABV also uses this pathway in nerve cells.

Even though RABV and both lineages of ABLV utilize clathrin-dependent endocytosis and early endosomes during cell entry, important differences exist in their *in vitro* cellular tropisms and reservoir hosts. Some cell lines that are resistant to ABLV infection are susceptible to RABV. The two lineages of ABLV also differ by 33 amino acids in the external domain of the glycoprotein (Weir *et al*. 2013). A study using ABLV glycoprotein-mediated vesicular stomatitis virus entry into a variety of human, bat, and embryonic cat tissues found a 6- to 45-fold difference in infectivity between the two ABLV lineages with several cell types being much less susceptible to ABLVp (Weir *et al*. 2013). Cell types in this study included those of neuronal and non-neuronal origin, including kidney, lung, skin, and ovarian cell lines. Infection mediated by ABLV glycoprotein appears to be at least partially related to adherence, since some cells were demonstrated to be susceptible in the adherent state, but not while in suspension. This study also showed that the anti-clumping agent dextran sulfate inhibits ABLV infectivity of the adherent cells, with ABLVp affected to a greater extent than ABLVs (Weir *et al*. 2013). Dextran sulfate also has antiviral effects against many other enveloped DNA and RNA viruses.

Further work is required to learn the pathway and receptor involved in the other lyssavirus' entry into target cells and whether the process is the same for other host cells and other host species. Primary cell cultures should also be utilized, as well as viruses derived from humans and different bat species before extensive passage *in vitro*. Determining the factors involved in host cell entry will aid in determining whether new lyssaviruses are able to infect human cells and, if so, which cells may be targeted. This may allow preventative measures to be targeted at the viral species of concern, the

reservoir hosts, and the route of transmission as well as to reduce concern about viral species which are not likely to infect or spread readily between people. The latter is important for economic considerations and bat conservation efforts.

The site of cellular infection is typically the neuromuscular junction, but RABV has been shown to disseminate via blood and enter the central nervous system in the hypothalamus at the neurovascular junction as well. Viruses are then transported back to the cell body by retrograde axonal transport (Weir *et al*. 2014a).

2.2.6 Prevention of lyssavirus infection

Prevention measures include mandatory vaccination of domestic dogs and cats. Avoidance of sick or unknown mammals or those acting in an unusual manner is suggested. Healthy domestic animals that have bitten a person should be quarantined for 10 days, while ill domestic animals and any wild animals should be euthanized and tested for the presence of lyssaviruses. In Latin America, a dog rabies reduction program dramatically decreased rabies infections while the US, Canada, and Europe have also utilized oral vaccination of wild carnivores, including the use of vaccine-laden bait for these rabies vectors which were important in controlling rabies in raccoons (Finley 1998; Heymann 2008).

Following close contact with bats (bite, scratch, or exposure to saliva), the wound should be thoroughly cleansed prior to administration of human RABV immunoglobulin and a four-step PEP vaccination. Pre-exposure vaccination is recommended for those involved in the treatment of animals, including those who care for bats; animal control personnel; cavers; those involved in field and laboratory mammal research; and long-time travelers to regions with a high incidence of rabies, especially since vaccines and immunoglobulin are scarce in some regions of Asia and Africa (Heymann 2008; Warrell & Warrell 2015). PEP should begin as soon as possible following exposure to saliva of a potentially rabid animal, except for those licked on intact skin. These prophylactic measures are also effective in preventing disease for those exposed to ABLV, but not humans or cats exposed to Mokula virus (Kgaldi *et al*. 2013; Francis *et al*. 2014). Of note, while RABV vaccination protected mice against intracerebral inoculation with ABLVp, a significant number of animals were not protected against intracranial (50% protection) or peripheral (79% protection) inoculation with ABLVs (Weir *et al*. 2014a), indicating lineage-dependent differences in protection against ABLV, and perhaps other lyssavirus subpopulations as well.

In a recent study of 270 southern Guatemalan households within 2 km of a cave housing unidentified species of bats, approximately 25% reported owning an animal bitten by bats or having a household member bitten. While less than 10% of those in the survey had completed primary school, over half of the population had vaccinated at least one of their animals against RABV and one-third had doors or windows that prevented bat entry (Moran *et al*. 2015). Awareness of bats as a source of rabies was, nevertheless, poor: while 71% knew that transmission was associated with animal bites, almost all of the people identified dogs as a source of rabies and about a quarter of the population identified cats, only 10% of those surveyed identified bats. Furthermore, approximately half of the responding households would seek medical attention if bitten or scratched by a bat: less than 75% would do so even if the bat was potentially rabid. Less than a quarter of the people said that they would seek PEP after a bite by a potentially rabid

animal and less than 10% would do so following a bat bite even though PEP is free and widely available for those bitten by dogs (Moran *et al*. 2015). While no bat-associated human rabies cases have been reported in Guatemala, hundreds of cattle rabies cases have occurred, primarily in northern regions of the country, demonstrating the possibility of transmission from bats to humans. Further studies focusing on the northern regions of the country could more clearly indicate the need for enhanced educational prevention programs for populations with little formal education in a region with many livestock rabies cases, particularly in light of the feeding patterns of vampire bats.

Control of vampire bat populations is challenging and has involved applying anticoagulants onto bats which, upon returning to their colonies, spread them to other animals, leading to their deaths. Oral vaccination of wildlife against RABV has also met with some success and may be more affordable (Johnson *et al*. 2010).

2.2.7 Immune response to lyssaviruses

2.2.7.1 Involvement of interferons in lyssavirus infection of bats Interferons are known to be vital to bat anti-viral defense. Pathogenic RABV reverse engineered to express interferon-γ (IFN-γ) was highly attenuated and had a 100-fold higher 50% lethal dose than non-altered RABV. This attenuation appears to result from early induction of IFN-α and IFN-β by IFN-γ (Barkhouse *et al*. 2014). The importance of IFN-α and IFN-β was shown by the loss of protection in animals lacking functional IFN receptors. This study opens up the possibility that delivery of IFN-γ to the brain may save the lives of RABV-exposed humans who might otherwise fail to survive. The role of T lymphocytes and IFN-γ in RABV immunity appears to outweigh the protection afforded by B lymphocytes and neutralizing antibodies.

In order to study the effects of other IFNs on lyssaviruses, IFN-ω and IFN-κ were expressed in an *E. serotinus* brain cell line. IFN-ω strongly activates IFN signaling as evidenced by increased ISG56, Mx1, and IFIT3 expression in response to stimulus. IFN-κ is a weaker activator than IFN-ω (He *et al*. 2014a). Recombinant bat IFN-ω suppressed replication of EBLV-1 in an *E. serotinus* brain cell line, but IFN-κ had no effect. Both recombinant IFN-ω and IFN-κ decreased RABV and EBLV-2 replication in brain cells to a lesser extent, with IFN-ω being more suppressive and active at lower concentrations. Interestingly, EBLV-1, EBLV-2, and RABV infection of brain cell lines did not alter production of IFN-ω or IFN-induced genes Mx1 and IFIT3, while IFN-κ expression was downregulated 50% by these lyssaviruses (He *et al*. 2014a).

2.2.7.2 The role of viral sensors of the innate immune response to lyssaviruses He *et al*. (2014b) used transfection with the SV40 *T* gene to establish five permanent cell lines from a single male bat of the European bat species *M. myotis* to aid in the study of infection and resistance to infection by various bat tissues by EBLV-1, EBLV-2, and RABV in their natural hosts. These studies had been previously complicated since all 52 European bat species are endangered and protected. The *M. myotis* cell lines were established from the cerebrum, nervus olfactorius, tonsil, nasal epithelium (all fibroblast-like, not neuronal), and the peritoneal cavity (epithelial-like) (He *et al*. 2014b). The cell lines expressed varying levels of the innate immune system's pathogen pattern recognition receptors and viral sensors TLR3, RIG-1, and MDA5, with the cerebral line expressing the lowest levels. Upon stimulation, the cells up-regulated

several IFN-inducible genes (ISG43, ISG56, Mx1, and IFIT3). TLR3 recognizes dsRNA while RIG-1 induces IFN in RABV-infected cells.

Most *M. myotis* cell lines were susceptible to infection by EBLV-1, EBLV-2, and RABV, but the cerebral line supported only a low level of replication of EBLV-1 and RABV (He *et al*. 2014b). Infection with RABV significantly up-regulated expression of the tested IFN-inducible genes in the cerebral line and to a greater extent than in other *M. mytois* cell lines, but had a far lesser degree of up-regulation of TLR3. The latter has been implicated in RABV pathology in human neurons by assisting in the formation of viral Negri bodies (Ménager *et al*. 2009). This brain cell line expresses several markers characteristic of microglia (CD14 and CD68), which are less susceptible to RABV than neurons (Ray *et al*. 1997).

2.2.7.3 The role of cytokines in lyssavirus infection In both RABV- and EBLV-2-infected brains, the inflammatory cytokines tumor necrosis factor-α and interleukin (IL)-6 are generated in addition to IFNs. RABV may enter the central nervous system and overwhelm it more rapidly than the other lyssaviruses, thus explaining the lack of perivascular cuffing and lymphocyte infiltration (Johnson *et al*. 2010). The inflammatory cytokine IL-1 β is also produced, activated, and secreted during RABV infection of mouse dendritic cells. Mice deficient in the IL-1 receptor have increased RABV pathogenicity (Lawrence *et al*. 2013).

2.2.8 Lyssavirus surveillance

Many studies rely upon passive surveillance of materials from dead bats. Using enhanced passive surveillance, Schatz *et al*. (2014a) were able to detect bat rabies in German federal states from which it had not previously been reported. However, since clinically healthy animals may carry viral RNA within their oropharyngeal cavities, active surveillance of healthy animals may yield a more accurate picture of the actual risk of transmission of lyssaviruses from asymptomatic bats to other hosts. A combination of enhanced passive and active surveillance of bats may help to identify new lyssaviruses, their reservoir bat species, and their geographical distribution as well as whether the new lyssaviruses are pathogenic to humans. It may also aid in bat conservation efforts by delineating which bat species may be involved in potential transmission and assessing the actual threat, or lack thereof, to humans and what types of preventative measures need to be taken. Understanding the rarity of transmission of rabies from bats to humans in the majority of the world may help to allay fear of and hostility to bats.

2.3 OTHER RHABDOVIRUSES

Members of the Rhabdovirus genus *Vesiculovirus* include the vesicular stomatitis virus, which causes fever and blister-like lesions on the mouth lining, tongue, nose, and lips in agricultural animals, such as cattle, horses, and pigs, and occasionally in humans. Infection with Chandipura and Isfahan viruses also results in severe, febrile, encephalitic illness in people in India, primarily children (Menghani *et al*. 2012). Little, however, is known about the diversity of vesiculoviruses or other genera of non-lyssavirus rhabdoviruses. In the Americas, a novel rhabdovirus, designated American bat vesiculovirus, was

found in postmortem lung and liver tissues from 5% of rabies-negative big brown bats (*Eptesicus fuscus*) ($n=60$) having a history of human contact (Ng *et al*. 2013). This species was found by metagenomic sequencing of RNA viruses and shares 41–49% amino acid identity with other vesiculoviruses.

2.3.1 The Kern Canyon serogroup of genus *Vesiculovirus*

The Kern Canyon serogroup contains many isolates from bats, including Kern Canyon virus, Kolente virus, Mount Eglon bat virus, Fikirini virus, and Oita virus. Kern Canyon virus was isolated from the Yuma myotis (*Myotis yumanensis*) (Ghedin *et al*. 2013) from the western US. Similar viruses from other animals include: Fukuoka virus from Japanese mosquitoes, midges, and cattle; Barur virus from an Indian rodent; Nkolbisson virus from Central African mosquitoes; Keuraliba virus from rodents in Senegal; and Nishimuro viruses from Japanese pigs (Ghedin *et al*. 2013; Blasdell *et al*. 2015). Blasdell has suggested grouping all of the above into a new genus, *Ledantevirus*.

Kolente virus was isolated from Jones's roundleaf bat (*Hipposideros jonesi*) and from a bat ectoparasite (*Ampblyomma* ticks) in Guinea. It leads to cytopathic effect in a kidney cell line and, upon intracranial inoculation of newborn mice, causes loss of balance, lethargy, and paralysis (Ghedin *et al*. 2013). Infection of adult, outbred mice is, however, asymptomatic. Kolente virus is antigenically related to the above ledanteviruses, with the exception of the Oita virus.

The Mount Elgon bat virus is a rhabdovirus isolated from the salivary glands of *Rhinolophus hildebrandti eloquens* in Kenya (Metselaar *et al*. 1969; Murphy *et al*. 1970; Ghedin *et al*. 2013; Kading *et al*. 2013). Experimental intranasal infection of very young mice with Mount Elgon bat virus leads to fatal encephalitis. Virus traveled to the brain via the olfactory nerve, where it multiplied to high levels in the presence of large amounts of interferon and the absence of virus neutralizing antibody. In slightly older mice, the virus only reached the olfactory bulbs, where it remained until neutralizing antibody entered the area. Resistant mice, in contrast, had no blood antibody or interferon in their brains or nasal mucosa, suggesting an effective local immune response (Patel 1979). It is not known if the virus is pathogenic for young or adult bats or humans.

Infectious Fikirini rhabdovirus was isolated from liver, lungs, kidneys, brain, intestines, and feces of a Kenyan *Hipposideros vittatus*, but not from an oral swab (Kading *et al*. 2013). It grows to high titers in Vero cells, causing plaques (Kading *et al*. 2013). Since *H. vittatus* guano is collected from caves, viral transmission to humans might occur via the fecal route. The absence of virus from oral swabs does not necessarily imply that transmission might not occur by this route as well, since the presence of other viruses in saliva is intermittent (Kading *et al*. 2013).

Oita virus was isolated from *Rhinolophus cornutus* from Japan. It infects neurons in both the central and peripheral nervous system and causes fatal encephalitis in intracerebrally inoculated suckling mice. It is not associated with human illness (Iwasaki *et al*. 2004). This virus shares traits of both the *Lyssavirus* and *Vesiculovirus* genera, producing enveloped virions in the plasma membrane, but antibodies against Oita virus do not cross-react with lyssaviruses. It also is disseminated via the hematogenous pathway, as are vesiculoviruses and ephemeroviruses, rather than the neural route, as are lyssaviruses.

Gossas virus was isolated from *Tadarida* free-tailed bats from Africa as well as pigs (Ghedin *et al*. 2013).

2.3.2 Kumasi rhabdovirus

A colony of straw-colored fruit bats (*E. helvum*) from a large city in Ghana was found to have a new species of the dimarhabdovirus supergroup of rhabdovirus, designated the Kumasi rhabdovirus (Binger *et al*. 2015). It was isolated in a mixture of spleen and kidney cells derived from *E. helvum* and could also be cultured in Vero as well as IFN-competent human and primate cells. A greater degree of cytopathic effect was seen in the Vero cells from African green monkeys than in the bat cell line, suggesting that the bat cells possibly contain a protective factor. Kumasi rhabdovirus was detected in splenic, but not brain, tissue of 5.1% of the bats, including ill animals ($n = 487$). Since virus infection of kidneys, gut, lungs, central nervous system, or salivary glands was not observed, transmission via excreta, the respiratory route, or saliva appears unlikely. Transmission among bats may occur via a blood-borne route due to the aggressive behavior of juvenile males in the breeding season, which corresponds to one of the wet seasons (discussed below).

Antibody prevalence in bats was 11.5% as determined by immunofluorescence and 6.4% had neutralizing antibodies. Prevalence was highest in the two annual wet seasons, especially in juvenile bats. A survey of 1240 local female mosquitoes of six genera (*Aedes, Culex, Eretmapodites, Lutzia, Mansonia*, and *Toxorhynchites*) was negative for this rhabdovirus. Antibodies were present in 28.9% of pig sera ($n = 107$), but not in sheep, goat, or cattle ($n > 100$ of each). Furthermore, antibodies were present in 11% of humans having occupational exposure to the bat colony ($n = 45$), while only 0.8% of unexposed people had seroconverted (Binger *et al*. 2015). Exposed workers were known to hunt and kill the bats. Phylogenetic analysis places Kumasi virus clusters with Mount Elgon virus, Oita rhabdovirus, and Kern Canyon virus. It has been suggested that the Kumasi and Oita rhabdovirus and Mount Elgon bat virus, belong to group C ledanteviruses. Nishimuro virus from pigs is in a sister group to this clade of bat-associated viruses (Binger *et al*. 2015).

2.3.3 Unclassified rhabdoviruses

Oropharyngeal swabs from bats in Spain were examined for the presence of rhabdovirus RNA (Aznar-Lopez *et al*. 2013). From these, nine unique rhabdovirus-related sequences were detected from 0.7% of bats ($n = 1488$) from five of twenty-seven tested bat species. The following viruses were isolated from the corresponding species of bat: *Eptesicus isabellinus* rhabdovirus 1–5, *Hypsugo savii* rhabdovirus 1, *Miniopterus schreibersii* rhabdovirus 1, *Plecotus auritus* rhabdovirus 1, and *Rhinolophus ferrumequinum* rhabdovirus 1. These sequences did not appear to constitute a monophyletic group, even those originating from the same species of bat, and are not related to any other group of rhabdoviruses. Additionally, when bat parasites were examined, all samples of nycteribiids (*Nycteribia kolenatii* from *M. daubentonii* bats and *Penicillidia conspicua* and *Nycteribia schmidli* from *M. schreibersii* bats) were positive for rhabdovirus RNA, while all three bat true bugs (*Cimex pipistrelli*) and bat ticks (*Argas vespertilionis*) were negative (Basak *et al*. 2007; Aznar-Lopez *et al*. 2013).

Rhabdovirus RNA was also seen in fecal swabs of *Chaerephon* species (1 of 35 bats) and *Miniopterus africanus* (1 of 9 bats) from Kenya. These rhabdoviruses were nearly identical to each other, but appear to be novel viral lineages based upon their

genetic distance (less than 85% nucleotide identity in highly conserved genomic regions) from other known rhabdoviruses (Conrardy *et al*. 2014).

2.4 CONCLUSIONS

Rhabdoviruses have a diverse host range that includes vertebrates, invertebrates, and plants throughout the world. Genera that affect bats include *Lyssavirus* and *Vesiculovirus*, in addition to unclassified rhabdoviruses. Some vesiculoviruses may cause mild to serious illness in humans or domestic animals, but none of these have been found in bats. By contrast, many lyssaviruses cause rabies in not only bats but also in humans. Rabies is almost universally fatal in the absence of vaccination, which may be administered post-infection soon after potential exposure.

While evolutionary studies suggest that many or all lyssaviruses originated in bats, in many of the regions of the world, human infection is primarily due to the bite of a canid or a felid. Bats, raccoons, skunks, and several other mammals also transmit lyssaviruses to humans. In regions that have largely eliminated transmission by domestic or wild canids, bat bites may be the leading cause of human infection, especially in Latin America, where vampire bats are the major rabies reservoir and vector for both human and cattle infection. Lyssaviruses found to infect bats are as follows: rabies virus, EBLV-I and EBLV-2, Bokeloh virus, Lleida bat lyssavirus, West Caucasian bat lyssavirus, Irkut virus, Aravan virus, Khujand virus, Lagos bat virus, Duvenhage virus, Shimoni virus, and Australian bat lyssavirus. Of these, rabies virus is responsible for the majority of human rabies cases worldwide; however, Duvenhage virus, EBLV-1 and -2, ABLV, and Irkut virus also may cause fatal disease in humans. Fortunately, most of these viruses have killed very small numbers of people. Bat transmission of rabies virus to humans only occurs in the Americas. In addition to the major vector, hematophagous vampire bats, rabies virus may also undergo zoonotic transmission from frugivorous and insectivorous bats.

Of the 333 bat species found in Latin America or the Caribbean, 22.5% have occasionally been found to be infected with rabies virus. The highest numbers of rabid bat species are found in Brazil and Mexico. All three hematophagous bat species may be infected by rabies virus, in addition to two of the three carnivorous bat species in the region. The non-migratory common vampire bat is the major reservoir of rabies virus in the Americas and caused nearly 100 human cases in 2004–2005 and many deaths in cattle. Nevertheless, nearly 90% of rabid bats are insectivorous, Molossidae family members. Within the bats' bodies, rabies virus has been detected in the following sites: brain, salivary glands, tongue, brown fat, lungs, heart, stomach, liver, spleen, urinary bladder, kidneys, and intestines as well as in the bats' feces.

Protection against lyssavirus infection of neurons and the subsequent pathology appears to rely heavily upon IFN-γ, IFN-α, IFN-β, and IFN-ω. The latter is not important in human anti-viral immunity but is very important in bats' defenses. Inflammatory cytokines also appear to be important, especially in protection against rabies virus.

Of the non-rabies virus lyssaviruses, EBLV-1 and EBLV-2 antibodies or RNA are commonly detected in some species of European bats, particularly *Eptesicus* and *Myotis* species, nevertheless, only two human cases of EBLV-1 and two human cases of EBLV-2 have been reported. Three cases of human rabies were due to infection with

Duvenhage virus in Africa as well as one human infection with Irkut virus in Eastern Siberia. There have been no reports of human infection by either the Lagos bat virus or Shimoni virus, although the former has led to fatal infection in rabies virus-vaccinated cats. A 2016 study in Sri Lanka reported a new bat lyssavirus, Gannoruwa bat lyssavirus, in 62 grounded bats, almost all of which succumbed to infection. A total of three human cases of infection with Australian bat lyssavirus have been reported. All three people developed encephalitic rabies prior to death.

A number of vesiculoviruses have been detected in bats, including the American bat vesiculovirus, Kern Canyon virus, Kolente virus, Mount Eglon bat virus, Fikirini virus, and Oita virus. Many of these cause severe disease in young, but not adult, mice. None of these are known to be human pathogens. Other unclassified rhabdoviruses have also been detected in bats. None of these are known to cause disease in humans.

Increasing active surveillance of healthy bats may help to identify new lyssaviruses or other rhabdoviruses, their reservoir bat species, and geographical distribution, as well as whether novel lyssaviruses are likely to be transmitted from bats to humans and whether or not any newly discovered viruses are pathogenic to humans. Since canids and felines are known to be the major rabies virus reservoir hosts in much of the world, studies may be more effective if attention was focused on these species in addition to or instead of on bats, especially due to the rarity of transmission of lyssaviruses from bats to humans outside of Latin America. Given the small numbers of people infected or killed by non-rabies virus rhabdoviruses, the large amounts of money required to perform such active surveillance efforts might be more profitably directed to other public health programs.

REFERENCES

Allendorf SD, Cortez A, Heinemann MB, Harary CMA, Antunes JMAP, Peres MG, Vicente AF, Sodré MM, da Rosa AR, Megid J. 2012. Rabies virus distribution in tissues and molecular characterization of strains from naturally infected non-hematophagous bats. *Virus Research.* 165:119–125.

Amengual B, Bourhy H, Lopez-Roig M, Serra-Cobo J. 2007. Temporal dynamics of European bat lyssavirus type 1 and survival of *Myotis myotis* bats in natural colonies. *PLoS ONE.* 2:1–7.

Aznar-Lopez C, Vazquez-Moron S, Marston DA, Juste J, Ibáñez C, Berciano JM, Salsamendi E, Aihartza J, Banyard AC, McElhinney L, Fooks AR, Echevarria J. 2013. Detection of rhabdovirus viral RNA in oropharyngeal swabs and ectoparasites of Spanish bats. *Journal of General Virology.* 94:69–75.

Banyard AC, Johnson N, Voller K, Hicks D, Nunez A, Hartley M, Fooks AR. 2009. Repeated detection of European bat lyssavirus type 2 in dead bats found at a single roost site in the UK. *Archives of Virology.* 154:1847–1850.

Barkhouse DA, Garcia SA, Bongiorno EK, Lebrun A, Faber M, Hooper DC. 2014. Expression of interferon gamma by a recombinant rabies vaccine strongly attenuates the pathogenicity of the virus via induction of type I interferon. *Journal of Virology.* 89(1):312–322.

Basak S, Mondal A, Polley S, Mukhopadhyay S, and Chattopadhyay D. 2007. Reviewing Chandipura: a vesiculovirus in human epidemics. *Bioscience Reports.* 27:275–298.

Binger T, Annan A, Drexler JF, Müller MA, Kallies R, Adankwah E, Wollny R, Kopp A, Heidemann H, Dei D, Agya-Yao FC, Junglen S, Feldt T, Kurth A, Oppong S, Adu-Sarkodie Y,

Drosten C. 2015. A novel rhabdovirus isolated from the straw-colored fruit bat *Eidolon helvum*, with signs of antibodies in swine and humans. *Journal of Virology.* 89:4588–4597.

Blasdell KR, Guzman H, Widen SG, Firth C, Wood TG, Holmes EC, Tesh RB, Vasilakis N, Walker PJ. 2015. Ledantevirus: a proposed new genus in the Rhabdoviridae has a strong ecological association with bats. *American Journal of Tropical Medicine and Hygiene.* 92(2):405–410.

Bourhy H, Kissa B, Lafon M, Sacramento D, Tordo N. 1992. Antigenic and molecular characterization of bat rabies virus in Europe. *Journal of Clinical Microbiology.* 30:2419–2426.

Calisher CH, Ellison JA. 2012. The other rabies viruses: the emergence and importance of lyssaviruses from bats and other vertebrates. *Travel Medicine and Infectious Diseases.* 10:69–79.

Casagrande DKA, da Cruz Favaro ABB, de Carvalho C, Picolo MR, Hernandez JCB, Lot MS, Albas A, Araújo DB, Pedro WA, Queiroz LH. 2014. Rabies surveillance in bats in Northwestern State of São Paulo. *Revista da Sociedade Brasileira de Medicina Tropical.* 47(6):709–715.

Condori-Condori RE, Streicker DG, Cabezas-Sanchez C, Velasco-Villa A. 2013. Enzootic and epizootic rabies associated with vampire bats, Peru. *Emerging Infectious Diseases*. 19(9):1463–1469.

Conrardy C, Tao Y, Kuzmin IV, Niezgoda M, Agwanda B, Breiman RF, Anderson LJ, Rupprecht CE, Tong S. 2014. Molecular detection of adenoviruses, rhabdoviruses, and paramyxoviruses in bats from Kenya. *American Journal of Tropical Medicine and Hygiene.* 91(2):258–266.

Davis A, Gordy P, Rudd R, Jarvis JA, Bowen RA. 2012. Naturally acquired rabies virus infections in wild-caught bats. *Vector Borne Zoonotic Diseases.* 12:55–60.

Dzikwi AA, Kuzmin II, Umoh JU, Kwaga JKP, Ahmad AA, Rupprecht CE. 2010. Evidence of Lagos bat virus circulation among Nigerian fruit bats. *Journal of Wildlife Diseases.* 46(1):267–271.

Escobar LE, Peterson AT, Favi M, Yung V, Medina-Vogel G. 2015. Bat-borne rabies in Latin America. *Revista do Instituto de Medicina Tropical de Sao Paulo.* 57(1):63–72.

Finley D. 1998. *Mad Dogs: The New Rabies Plague*. Texas A&M University Press: College Station, TX.

Francis JR, McCall BJ, Hutchinson P, Powell J, Vaska VL, Nours C. 2014. Australian bat lyssavirus: implications for public health. *The Medical Journal of Australia.* 201(11):647–649.

Freuling CM, Abendrogh B, Beer M, Fischer M, Hanke D, Hoffmann B, Höper D, Just F, Mettenleiter TC, Schatz J, Müller T. 2013. Molecular diagnostics for the detection of Bokeloh bat lyssavirus in a bat from Bavaria, Germany. *Virus Research*. 177:201–204.

Gautret P, Harvey K, Pandey P, Lim PL, Leder K, Piyaphanee W, Shaw M, McDonald SC, Schwartz E, Esposito DH, Parola P, and the GeoSentinal Network. 2015. Animal-associated exposure to rabies virus among travelers, 1997–2012. *Emerging Infectious Diseases.* 21(4):569–577.

Ghedin E, Rogers MB, Widen SG, Guzman H, da Rosa APAT, Wood TG, Fitch A, Popov V, Holmes EC, Walker PJ, Vasilakis N, Tesh RB. 2013. Kolente virus, a rhabdovirus species isolated from ticks and bats in the Republic of Guinea. *Journal of General Virology.* 94:2609–2615.

Gunawardena PS, Marston DA, Ellis RJ, Wise WL, Karawita AC, Breed AC, McElhinney LM, Johnson N, Banyard AC, Fooks AR. 2016. Lyssavirus in Indian flying foxes, Sri Lanka. *Emerging Infectious Diseases.* 22(8):456–459.

He X, Korytář T, Schatz J, Freuling CM, Müller T, Köllner B. 2014a. Anti-lyssaviral activity of interferons κ and ω from the serotine bat, *Eptesicus serotinus*. *Journal of Virology.* 8(10):5444–5454.

He X, Korytář T, Zhu Y, Pikula J, Bandouchova H, Zuhal J. 2014b. Establishment of *Myotis myotis* cell lines – model for investigation of host-pathogen interaction in a natural host for emerging viruses. *PLoS ONE.* 9(10):109795.

Heymann DL (ed.). 2008. Rabies. In: *Control of Communicable Diseases Manual*, 19th edition. APHA Press: Washington, DC, pp. 499–508.

Iwasaki T, Inoue S, Tanaka K, Sato Y, Morikawa S, Hayasaka D, Moriyama M, Ono T, Kanai S, Yamada A, Kurata T. 2004. Characterization of Oita virus 296/1972 of Rhabdoviridae isolated from a horseshoe bat bearing characteristics of both lyssavirus and vesiculovirus. *Archives of Virology.* 149(6):1139–1154.

Jackson AC, Ye HT, Phelan CC, Ridaura-Sanz C, Zheng Q, Li ZS, Wan XQ, Lopez-Cerella E. 1999. Extraneural organ involvement in human rabies. *Laboratory Investigations.* 79:945–951.

Jackson AC, Ye H, Ridaura-Sanz C, Lopez-Corella E. 2001. Quantitative study of the infection in brain neurons in human rabies. *Journal of Medical Virology.* 65:614–618.

Johnson N, Phillipotts R, Fooks, AR. 2006. Airborne transmission of lyssaviruses. *Journal of Medical Microbiology.* 55:785–790.

Johnson N, Vos A, Freuling C, Tordo N, Fooks AR, Müller T. 2010. Human rabies due to lyssavirus infection of bat origin. *Veterinary Microbiology.* 142:151–159.

Kading RC, Gilbert AT, Mossel EC, Crabtree MB, Kuzmin IV, Niezgoda M, Agwanda B, Markotter W, Weil MR, Montgomery JM, Rupprecht C, Miller BR. 2013. Isolation and molecular characterization of Fikirini rhabdovirus, a novel virus from a Kenyan bat. *Journal of General Virology.* 94: 2393–2398.

Kgaldi J, Wright N, Coertse J, Markotter A, Marston D, Fooks AR, Freuling CM, Sabeta CT, Nel LH. 2013. Diversity and epidemiology of Mokola virus. *PLoS Neglected Tropical Diseases.* 7(10):e2511.

Lawrence TM, Hudacek AW, de Zoete MR, Flavell RA, Schnell MK. 2013. Rabies virus is recognized by the NLRP3 inflammasome and activates interleukin-1β release in murine dendritic cells. *Journal of Virology.* 87(10):5848–5857.

López-Roig M, Bourhy H, Lavenir R, Serra-Cobo J. 2014. Seroprevalence dynamics of European bat *Lyssavirus* type I in a multispecies bat colony. *Viruses.* 6:3386–3399.

Markotter W. 2007. Molecular epidemiology and pathogenesis of Lagos bat virus: A rabies related virus specific to Africa. PhD thesis. University of Pretoria, South Africa.

McElhinney LM, Marston DA, Leech S, Freuling CM, van der Poel WHM, Echevarria J, Vázquarez-Morón S, Horton DL, Müller T, Fooks AR. 2011. Molecular epidemiology of bat lyssaviruses in Europe. *Zoonoses and Public Health.* 60:35–45.

Megali A, Yannic G, Zahno M-L, Brügger D, Bertoni G, Christe P, Zanoni R. 2010. Surveillance for European bat lyssavirus in Swiss bats. *Archives of Virology.* 155:1655–1662.

Ménager P, Roux P, Mégret F, Bourgeois JP, Le Sourd AM, Danckaert A, Lafage M, Préhaud C, Lafon M. 2009. Toll-like receptor 3 (TLR3) plays a major role in the formation of rabies virus Negri Bodies. *PLoS Pathology.* 5(2):e1000315.

Menghani S, Chikhale R, Raval A, Wadibhasme P, Khedekar P. 2012. Chandipura virus: an emerging tropical pathogen. *Acta Tropica*. 124:1–14.

Metselaar D, Williams MC, Simpson DI, West R, Mutere FA. 1969. Mount Elgon bat virus: a hitherto undescribed virus from *Rhinolophus hildebrandtii eloquens K. Anderson. Archiv für die gesamte Virusforschung.* 26(1):183–193.

Moran D, Juliao P, Alvarez D, Lindblade KA, Ellison JA, Gilbert AT, Petersen B, Rupprecht C, Recuenco S. 2015. Knowledge, attitudes and practices regarding rabies and exposure to bats in two rural communities in Guatemala. *BMC Research Notes*. 8:955–961.

Murphy FA, Shope RE, Metselaar D, Simpson DI. 1970. Characterization of Mount Elgon bat virus, a new member of the rhabdovirus group. *Virology.* 40(2):288–297.

Ng TF, Driscoll C, Carlos MP, Prioleau A, Schmieder R, Dwivedi B, Wong J, Cha Y, Head S, Breitbart M, Delwart E. 2013. Distinct lineage of vesiculovirus from big brown bats, United States. *Emerging Infectious Diseases.* 19(12):1978–1980.

Nolden T, Banyard AC, Finke S, Fooks AR, Hanke D, Höper D, Horton DL, Mettenleiter TC, Müller T, Teifke JP, Freuling CM. 2014. Comparative studies on the genetic, antigenic and pathogenic characteristics of Bokeloh bat lyssavirus. *Journal of General Virology.* 95:1647–1653.

Patel JR. 1979. The pathogenesis of infection in mouse brain by Mount Elgon bat virus. *Journal of General Virology*. 45(3):591–598.

Picard-Meyer E, Robardet E, Arthur L, Larcher G, Harbusch C, Servat A, Cliquet F. 2014. Bat rabies in France: A 24-year retrospective epidemiological study. *PLoS ONE*. 9(6):e98622.

Ray NB, Power C, Lynch WP, Ewalt LC, Lodmell DL. 1997. Rabies viruses infect primary cultures of murine, feline, and human microglia and astrocytes. *Archives of Virology.*142(5):1011–1019.

Reynes J-M, Molia S, Audry L, Hout S, Ngin S. Walston J Bourhy H. 2004. Serologic evidence of lyssavirus infection in bats, Cambodia. *Emerging Infectious Diseases.* 10(12):2231–2234.

Schatz J, Fooks, McElhinney L, Horton D, Echevarria J, Vázquez-Moron S, Kooi EA, Rasmussen TB, Müller T, Freuling CM. 2012. Bat rabies surveillance in Europe. *Zoonoses and Public Health.* 60:22–34.

Schatz J, Freuling CM, Auer E, Goharriz H, Harbusch C, Johnson N, Kaipf I, Mettenleiter TC, Mühldorter K, Mühle R-U, Ohlendorf B, Pott-Dörfer B, Prüger J, Ali HS, Stiefel D, Teubner J, Ulrich RG, Wibbelt G, Müller T. 2014a. Enhanced passive bat rabies surveillance in indigenous bat species from Germany – A retrospective study. *PLoS ONE Neglected Tropical Diseases.* 8(5):e2835.

Schatz J, Teifke JP, Mettenleiter TC, Aue A, Stiefel D, Müller T, Freuling CM. 2014b. Lyssavirus distribution in naturally infected bats from Germany. *Veterinary Microbiology.* 169:33–41.

Smith KA. 2012. Louis Pasteur, the father of immunology? *Frontiers in Immunology*. 3:Article 68.

Vázquarez-Morón S, Juste J, Ibáñez C, Ruiz-Villamor E, Avellón A, Vera M, Echevarría JE. 2008. Endemic circulation of European bat lyssavirus type I in serotine bats, Spain. *Emerging Infectious Diseases.* 14:1263–1266.

Viera LFP, Pereira S, Galante, AC, Castilho JG, Loiveria RN, Brandae PE, Kotait I. 2011. Detection of rabies virus nucleoprotein-RNA in several organs outside the central nervous system in naturally-infected vampire bats. *Pesquisa Veterinária Brasileira.* 31:922–925.

Voloch CN, Capellão RT, Mello B, Schrago CG. 2014. Analysis of adaptive evolution in *Lyssavirus* genomes reveals pervasive diversifying selection during species diversification. *Viruses.* 6:4465–4478.

Warrell MJ, Warrell DA. 2015. Rabies: the clinical features, management and prevention of the classic zoonosis. *Clinical Medicine.* 15(1):78–81.

Weir DL, Annand EJ, Reid PA, Broder CC. 2014a. Recent observations on Australian bat lyssavirus tropism and viral entry. *Viruses.* 6:909–926.

Weir DL, Laing ED, Smith IL, Wang L-F, Broder CC. 2014b. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. *Virology Journal.* 11:40.

Weir DL, Smith IL, Bossart KN, Wang L-F, Broder CC. 2013. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. *Virology.* 444:21–30.

3

HENIPAVIRUSES AND OTHER PARAMYXOVIRUSES OF BATS

3.1 INTRODUCTION TO PARAMYXOVIRIDAE

The Paramyxoviridae family consists of enveloped, ssRNA (−) viruses with pleomorphic virions whose diameters average 500 nm, but have a wide size range. Their genomes are large (18 000 nucleotides) and contain six genes. The family is divided into the Pneumovirinae and Paramyxovirinae subfamilies. The Pneumovirinae subfamily includes human respiratory syncytial virus, the most common microbe responsible for airway and lung infections in infants and young children. It typically causes only mild, cold-like symptoms, but may lead to serious difficulty in breathing in very young babies.

The subfamily Paramyxovirinae contains the genera *Henipa-, Respiro-*, *Morbilli-*, *Rubula-*, *Avula-*, and *Jeilongvirus* in addition to some currently unclassified viruses. Members of Paramyxovirinae are associated with bat species throughout the world (Table 3.1). Henipaviruses are responsible for life-threatening illnesses in humans, horses, and pigs, primarily in Oceania and Southeast Asia. Morbilliviruses are the causative agents of human measles, canine distemper, and ruminant Rinderpest. Rubulaviruses include human mumps virus and parainfluenzaviruses 2 and 4.

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TABLE 3.1 Paramyxoviruses associated with bats

Bat family	Bat common name	Bat species	Virus
Pteropodidae	Sulawesi fruit bat	*Acerodon celebensis*	Henipavirus
Rhinolophidae	Heart-nosed bat	*Cardioderma cor*	Paramyxovirus
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	Morbillivirus
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	Morbillivirus
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	Rubulavirus
Emballonuroidea	Lesser free-tailed bat	*Chaerephon leucogaster*	Morbillivirus-related virus
Emballonuroidea	African sheath-tailed bat	*Coleura afra*	Morbillivirus
Emballonuroidea	African sheath-tailed bat	*Coleura afra*	Belinga bat virus
Emballonuroidea	White-bellied sheath-tailed bat	*Coleura kibomalandy*	Morbillivirus-related virus
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Nipah virus
Pteropodidae	Greater short-nosed bat	*Cynopterus sphinx*	Nipah virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Rubulavirus
Pteropodidae	None	*Dobsonia andersoni*	Hendra virus
Pteropodidae	Bare-backed fruit bat	*Dobsonia magna*	Hendra virus
Pteropodidae	Moluccan naked-backed fruit bat	*Dobsonia moluccense*	Hendra virus
Pteropodidae	Malagasy fruit bats	*Eidolon dupreanum*	Nipah virus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Achimota virus 1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Achimota virus 2
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	GH-M74a
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Henipa-like virus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Pneumovirus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Rubulavirus
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	Nipah virus
Vespertilionidae	Hottentot bat	*Eptesicus hottentotus*	Morbillivirus-related virus
Pteropodidae	Gambian epauletted fruit bat	*Epomophorus gambianus*	Nipah virus
Pteropodidae	East African epauletted fruit bat	*Epomophorus minimus*	Rubulavirus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Morbillivirus
Rhinolophidae	Aba roundleaf bat	*Hipposideros abae*	Morbillivirus
Rhinolophidae	Great roundleaf bat	*Hipposideros armiger*	Jeilongvirus

Rhinolophidae	Sundevall's roundleaf bat	*Hipposideros caffer*	Morbillivirus
Rhinolophidae	Noack's roundleaf bat	*Hipposideros caffer ruber*	Moribillivirus
Rhinolophidae	Noack's roundleaf bat	*Hipposideros caffer ruber*	Rubulavirus
Rhinolophidae	Ashy roundleaf bat	*Hipposideros cineraceus*	Jeilongvirus
Rhinolophidae	Giant roundleaf bat	*Hipposideros gigas*	Morbillivirus
Rhinolophidae	Giant roundleaf bat	*Hipposideros gigas*	Rubulavirus
Rhinolophidae	Sooty roundleaf bat	*Hipposideros fuliginosus*	Morbillivirus-related virus
Pteropodidae	Intermediate roundleaf bat	*Hipposideros larvatus*	Nipah virus
Pteropodidae	Pomona roundleaf bat	*Hipposideros pomona*	Nipah-like virus
Pteropodidae	Hammerhead bat	*Hypsignathus monstrosus*	Henipavirus
Pteropodidae	Hammerhead bat	*Hypsignathus monstrosus*	Rubulavirus
Vespertilionidae	Damara woolly bat	*Kerivoula argentata*	Morbillivirus-related virus
Pteropodidae	Woermann's bat	*Megaloglossus woermanni*	Rubulavirus
Miniopteridae	Montagne d'Ambre long-fingered bat	*Miniopterus cf. ambohitrensis*	Morbillivirus-related virus
Miniopteridae	Glen's long-fingered bat	*Miniopterus gleni*	Morbillivirus-related virus
Miniopteridae	None	*Miniopterus griveaudi*	Morbillivirus-related virus
Miniopteridae	Greater long-fingered bat	*Miniopterus inflatus*	Rubulavirus
Miniopteridae	None	*Miniopterus mahafaliensis*	Morbillivirus-related virus
Miniopteridae	Least long-fingered bat	*Miniopterus minor*	Morbillivirus-related virus
Miniopteridae	Natal long-fingered bat	*Miniopterus natalensis*	Paramyxovirus
Miniopteridae	Sororcula long-fingered bat	*Miniopterus sororculus*	Morbillivirus-related
Miniopteridae	Long-fingered bats	*Miniopterus* spp.	Nipah-like virus
Molossidae	Malagasy white-bellied free-tailed bat	*Mops leucostigma*	Morbillivirus-related virus
Molossidae	Midas bat	*Mops midas*	Moribillivirus-related virus
Mormoopidae	Natal free-tailed bat	*Mormopterus acetabulosus*	Morbillivirus-related virus
Molossidae	Peter's wrinkle-tipped bat	*Mormopterus jugularis*	Morbillivirus-related virus
Pteropodidae	Little collared fruit bat	*Myonycteris torquata*	Henipavirus
Vespertilionidae	Alcathoe myotis	*Myotis alcathoe*	Morbillivirus
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	Morbillivirus

(*Continued*)

TABLE 3.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Vespertilionidae	Long-fingered bat	*Myotis capaccinii*	Morbillivirus
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	Morbillivirus
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	Nipah-like virus
Vespertilionidae	Malagasy mouse-eared bat	*Myotis goudoti*	Morbillivirus-related virus
Vespertilionidae	Large mouse-eared bat	*Myotis myotis*	Morbillivirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Jeilongvirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Morbillivirus
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Nipah-like virus
Vespertilionidae	Banana bat	*Neoromicia nanus*	Morbillivirus-related virus
Vespertilionidae	Common noctule	*Nycteris thebaica*	Morbillivirus-related virus
Molossidae	Malagasy giant mastiff bat	*Otomops madagascariensis*	Morbillivirus-related virus
Molossidae	Large-eared free-tailed bat	*Otomops martiensseni*	Morbillivirus-related virus
Rhinonycteridae	Trouessart's trident bat	*Paratriaenops furculus*	Morbillivirus-related virus
Vespertilionidae	Banana pipistrelle	*Pipistrellus cf nanus*	Morbillivirus
Vespertilionidae	African pipestrelle	*Pipistrellus hesperidus*	Morbillivirus-related virus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Jeilongvirus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Henipavirus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Morbillivirus
Pteropodidae	Admiralty flying fox	*Pteropus admiralitatum*	Hendra virus
Pteropodidae	Black flying fox	*Pteropus alecto*	Hendra virus
Pteropodidae	Black flying fox	*Pteropus alecto*	Menangle virus
Pteropodidae	Bismarck masked flying fox	*Pteropus capistratus*	Hendra virus
Pteropodidae	Spectacled flying fox	*Pteropus conspicillatus*	Hendra virus
Pteropodidae	Spectacled flying fox	*Pteropus conspicillatus*	Menangle virus
Pteropodidae	Indian flying fox	*Pteropus giganteusi*	Nipah virus
Pteropodidae	Variable flying fox	*Pteropus hypomelanus*	Hendra virus
Pteropodidae	Variable flying fox	*Pteropus hypomelanus*	Nipah virus-MY
Pteropodidae	Variable flying fox	*Pteropus hypomelanus*	Tioman virus

Pteropodidae	Lyle's flying fox	*Pteropus lylei*	Nipah virus-MY
Pteropodidae	Lyle's flying fox	*Pteropus lylei*	Nipah virus-BD
Pteropodidae	Great flying fox	*Pteropus neohibernicus*	Hendra virus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalis*	Cedar virus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalus*	Geelong paramyxovirus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalus*	Hendra virus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalus*	Menangle virus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalus*	Teviot virus
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalus*	Yara Bend paramyxovirus
Pteropodidae	Madagascan flying fox	*Pteropus rufus*	Morbillivirus-related virus
Pteropodidae	Madagascan flying fox	*Pteropus rufus*	Nipah virus
Pteropodidae	Madagascan flying fox	*Pteropus rufus*	Tioman virus
Pteropodidae	Little red flying fox	*Pteropus scapulatus*	Hendra virus
Pteropodidae	Large flying fox	*Pteropus vampyrus*	Nipah virus-MY
Pteropodidae	Flying foxes	*Pteropus* sp.	Cedar virus
Pteropodidae	Flying foxes	*Pteropus* sp.	Hervey virus
Pteropodidae	Flying foxes	*Pteropus* sp.	Grove virus
Pteropodidae	Flying foxes	*Pteropus* sp.	Menangle virus
Pteropodidae	Flying foxes	*Pteropus* sp.	Teviot virus
Pteropodidae	Flying foxes	*Pteropus* sp.	Yeppoon virus
Rhinolophidae	Intermediate horse-shoe bat	*Rhinolophus affinis*	Nipah-like virus
Rhinolophidae	Dent's horseshoe bat	*Rhinolophus denti*	Morbillivirus-related virus
Rhinolophidae	Lander's horseshoe bat	*Rhinolophus landeri*	Morbillivirus-related virus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Nipah-like virus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Henipavirus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Rubulavirus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Sosuga Virus
Pteropodidae	Geoffroy's rousette	*Rousettus amplexicaudatus*	Nipah virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Bat parainfluenza virus

(*Continued*)

TABLE 3.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Nipah-like virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Tuhoko virus 1
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Tuhoko virus 2
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Tuhoko virus 3
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	Rubulavirus
Pteropodidae	Madagascan rousette	*Rousettus madagascariensis*	Henipavirus sp.
Pteropodidae	Madagascan rousette	*Rousettus madagascariensis*	Tioman virus
Vespertilionidae	Asiatic lesser yellow house bat	*Scotophilus kuhlii*	Nipah virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	Mapuera virus
Emballonuroidea	Black-bearded tomb bat	*Taphozous melanopogon*	Jeilongvirus
Emballonuroidea	Tomb bats	*Taphozous* sp.	Morbillivirus-related virus
Hipposideridae	Trident bat	*Triaenops afer*	Morbillivirus-related virus
Hipposideridae	Rufous trident bat	*Triaenops menamena*	Morbillivirus-related virus

3.2 DISEASES ASSOCIATED WITH PARAMYXOVIRIDAE

3.2.1 Henipaviruses and disease

The *Henipavirus* genus includes Hendra (HeV) and Nipah viruses (NiV), two pathogens with high mortality rates in humans and several agricultural animals. While many of the members of the *Henipavirus* genus have not been assigned to membership in either of the designated species, approximately 70 times more NiV infections have been reported than HeV infections. The genomic similarity between HeV and NiV is greater than 77%. HeV infections in humans primarily cause an influenza-like illness, which often progresses to severe pneumonia and death in 57% of those infected, while NiV infection typically leads to multifocal encephalitis in humans with a higher mortality rate (75%). Both viruses produce pathological alterations in humans characterized by disseminated, multi-organ vasculopathy, including endothelial infection and ulceration, vasculitis leading to thrombosis and occlusion, endothelial syncytia, parenchymal ischemia and microinfarction, as well as infection of parenchymal cells of the central nervous system, lung, kidneys, and other organs. Vasculopathy is most severe in the central nervous system, often in the presence of discrete necrotic or vacuolar, plaque-like lesions. Henipavirus-associated encephalitis may relapse years after the acute infection (Ong 2015).

3.2.2 Morbilliviruses and disease

The Morbilliviruses genus of paramyxoviruses is responsible for several potentially serious diseases. While measles can lead to severe disease or death in humans, it is usually a mild illness characterized by fever, persistent dry cough, runny nose, sore throat, inflamed eyes, Koplik's spots (tiny spots in the lining of the cheek), and a skin rash composed of large, flat blotches. The severe form of measles typically occurs in young children, whose fever may reach as high as 40–41 °C (104–105.8 °F), encephalitis, deafness, and pneumonia (Mayo Clinic 2016). Measles are transmitted between people by inhalation or aerosolized viral particles.

Canine distemper is often severe or fatal in dogs, but distemper is also naturally found in other domestic or peridomestic animals, including cats and raccoons. Symptoms of severe disease include: encephalomyelitis (an inflammation of the brain and spinal cord); ataxia; increased sensitivity to sensory stimuli; muscle twitching or spasm; paralysis; mental deterioration; loss of motor skills; and seizures (Health Comunities.com 2016). Virus is shed in bodily excretions and is usually transmitted by the airborne route. Distemper is asymptomatic in humans.

Rinderpest infected cattle and other species of even-toed ungulates, such as buffaloes, deer, large antelopes, and occasionally sheep and goats. Its symptoms included fever, necrotic inflammation of the mouth and lips, gastroenteritis, and lymphoid necrosis. The mortality rate in infected animals was high. During epidemics, it was the most lethal plague of cattle. The virus was shed in nasal and ocular excretions and transmitted by close contact with infected animals. Rinderpest is one of the very rare diseases that has been eradicated from nature (Merck Veterinary Manual 2016).

3.2.3 Rubulaviruses and disease

Mumps is a highly contagious disease whose symptoms are generally mild and include fever, headache, muscle ache, loss of appetite, and inflammation of the parotid salivary glands, inferior to the ears. Mumps may rarely result in complications, including meningitis and orchitis (CDC 2016). The virus is transmitted person-to-person via contact with saliva or mucus from the mouth, nose, or throat of an infected person.

Parainfluenza viruses may cause upper or lower respiratory infections, such as pneumonia in humans. Symptoms also include croup, bronchiolitis, and bronchitis. Disease may be severe or life-threatening in infants (MedLine Plus 2016). Transmission is human-to-human via the airborne route or by contact with infected surfaces.

3.3 HENIPAVIRUSES IN BATS

The genus *Henipavirus* contains two highly pathogenic viruses, Hendra and Nipah, discovered in 1994 in Australia and 1998 in Malaysia, respectively, with continuing, periodic outbreaks of NiV in India and Bangladesh (reviewed in Drexler 2009). In addition to humans, HeV and NiV cause life-threating illnesses in horses and pigs, respectively.

Other than mild, focal vasculitis, HeV has not been found to cause illness in bats. In horses, however, it causes severe respiratory and neurological disease as well as facial swelling, ataxia, and copious frothy nasal discharge containing infectious virus prior to death (reviewed by Middleton & Weingartl 2012). At least one dog was infected with HeV during the 2011 outbreak in Australia (reviewed by Middleton & Weingartl 2012).

Hendra and Nipah viruses are able to overcome species barriers and enter into horse or pig populations, respectively, and, from these amplifying hosts, into human populations without adaptation. In addition to reproducing in vascular and neuronal tissues, henipaviruses also replicate in bronchiolar and renal epithelium, thus allowing viral transmission via nasopharyngeal secretions, including saliva, and urine. NiV may be transferred from the pteropid bats to humans via palm sap contaminated by infected bat urine (described below). Due to their pathogenicity and ability to enter directly into humans, these viruses are classified as Biosafety Level 4 pathogens. In addition to causing severe acute disease, both species of henipaviruses have been shown to undergone periods of latency as well as recrudescence in humans (reviewed by Breed *et al*. 2011).

As of 2015, more than twenty henipa-like paramyxoviruses have been reported over a wide geographical range that includes Asia, Oceania, Africa, and Central America. Although bats appear to be the major reservoir for henipaviruses that are most pathogenic in humans, other groups of animals are believed to serve as reservoirs for other zoonotic henipaviruses, including the Mojiang virus from rats that has caused severe pneumonia in humans, including three known deaths (reviewed by Lee *et al*. 2015).

3.3.1 Henipaviruses in bats from Oceania and Southeast Asia

It had been thought that NiV was found only to the west of a major biogeographic barrier, Wallace's Line, while HeV was found only to its east. Breed *et al*. (2013),

however, established the presence of NiV east of Wallace's Line in East Timor. Wallace's Line runs between Southeast Asia in the west and the Australo-Papuan and Wallacean region in the east. Different groups of terrestrial vertebrates and invertebrates exist on either side of the line. Among terrestrial mammals, only rodents and bats are present on both sides of the line, being found from Southeast Asia to Australia. Thirteen species of Pteropodidae fruit bats occur only west of Wallace's Line, 67 species occur only to the east, and 20 species inhabit regions on both sides of the line (reviewed by Breed *et al*. 2013). The distribution of NiV appears to be dependent on the presence of specific fruit bat species, particularly *Pteropus vampyrus*.

During a 2014 henipavirus outbreak in the southern Philippines, 17 humans and 10 horses developed severe illness (11 cases of acute encephalitis syndrome, 5 with influenza-like illness, and 1 with meningitis). The fatality rate among those with acute encephalitis syndrome was 82% and both survivors developed residual severe neuromuscular disease. Five people are believed to have been infected by contact with an infected person and, more often, by either slaughtering or eating infected horse meat. Four cats also died soon after eating horse meat. Patients' sera contained neutralizing antibodies against NiV and lower titer antibodies against HeV. A short segment of viral RNA had 94–99% nucleotide identity to NiV isolates from Bangladesh and Malaysia (Ching *et al*. 2015).

In a study of paramyxovirus seroprevalance in bats from Papua New Guinea, 50% were positive for HeV and 55% for NiV henipaviruses as well as 38% positive for Tioman and 56% for Menangle rubulavirus ($n=66$). Additionally, 36% of tested bats produced antibodies to both types of paramyxoviruses, suggesting dual or sequential infection (Breed *et al*. 2010).

3.3.2 Henipaviruses and bats from Africa

Between 2008 and 2011, 8% of spleen samples from *Eidon helvum* in Zambia, during a 4-year study ($n=312$), contained paramyxovirus RNA. The positive samples were composed of seven novel paramyxoviruses, five of which were related to Nipah virus (73% nucleotide homology) in Henipavirus Group A, which contains several Zambian Nipah virus strains as well as some Hendra viruses. Henipavirus Group B is composed of a cluster of Zambian strains related to an unclassified Bat PV from Ghana and Cedar virus, another henipavirus. The other two viruses in the above study were related to unclassified bat paramyxoviruses from Ghana (74% nucleotide homology) and Congo Brazzaville (Muleya *et al*. 2014).

A study of sera from archived *E. helvum* bats ($n=44$) and humans ($n=497$) from Cameroon found NiV cross-neutralizing antibodies in 48% of bat and 3–4% of human samples. Seropositive human sera also neutralized HeV and live NiV. Almost all of those who were seropositive butchered bats for bushmeat. Butchering bats and living in areas with little forest cover, as is found in open savannah lands or areas undergoing deforestation, were found to be the most significant risk factors for the development of neutralizing antibodies (Pernet *et al*. 2014). It is important to note that in 2010, HIV-1 prevalence in Cameroon was estimated to be 5% by UNAIDS, only slightly above that seen in the Cameroon henipavirus isolates. Unlike NiV from Asia or Australia, however, no henipavirus-related encephalitis has been reported in Africa as of 2013. This may be due to misdiagnosis or that the African henipa-like viruses may be nonpathogenic to humans, similar to the lack of illness in those infected by Cedar virus (described below).

The size and density of reservoir host populations help to determine the ability of species or its subgroups to maintain viruses which cause acute or immunizing infections. Typically, such microbes require either large host population sizes or a very high birth rate in order to maintain sufficient numbers of susceptible individuals to maintain transmission. High host population density also aids in microbial maintenance. Interestingly, antibodies to henipaviruses were detected in a remote subspecies of *E. helvum annobonensis* ($n = 73$) on the very small Annobón Island, part of a volcanic island chain in the Gulf of Guinea that has never been connected to continental Africa or to the other three islands in this island chain (Peel *et al*. 2012). Seroprevalance to NiV was higher than that to HeV and younger bats typically had lower titers than adult bats. No differences were seen between the sexes (Peel *et al*. 2012). This was unexpected since the Annoón bat population was isolated from other bat populations and thought to be too small to maintain viruses that cause acute, immunizing infections, despite the habit of this species of bat to form very large seasonal colonies throughout mainland Sub-Saharan Africa. The entire population size of *E. helvum* on Annobón Island, however, is believed to be 1600–2500 individuals. It is possible that the henipaviruses in these bats undergo recrudescence (Peel *et al*. 2012).

3.3.3 Henipaviruses in bats from Madagascar

In Madagascar, 2.3% of serum samples from *Pteropus rufus* and 19.2% of *Eidolon dupreanum* produced cross-reactive anti-henipavirus antibodies (Iehlé *et al*. 2007). The various Malagasy fruit bats share ecological niches, eating or roosting in the same locales (reviewed by Pernet *et al*. 2014). *Eidolon* species are long-distance fliers and are present throughout Sub-Saharan Africa, so lateral transfer of henipaviruses could occur between *Eidolon* species on mainland Africa and Madagascar.

3.3.4 Henipavirus proteins and infection of bats

The G and F henipavirus surface glycoproteins are responsible for host cell tropism. The G proteins bind to cellular ephrinB2 or B3 receptors and the fusion-active (cleaved) F proteins are required for fusion and entry into host target cells as well as causing fusion with uninfected cells and syncytia formation. G and F proteins from an exogenous African henipavirus (strain M74) induce syncytium formation in a kidney cell line from *Hypsignathus monstrosus* bats, but not in nonbat kidney cells. Syncytia are also found in two other cell lines from *H. monstrosus* and *E. helvum* fruit bats when the viruses coexpressed the M74 glycoproteins. The G protein is transported from the endoplasmic reticulum to transfected bat cell surfaces, while cell surface expression was only found in a small fraction of cell lines derived from other mammalian species. The G protein of NiV, however, is transported efficiently in both bat and other mammalian cells. In bat cells, the M74 G protein is primarily expressed in the endoplasmic reticulum, consistent with the finding that all N-glycans of M74-G proteins are of the mannose-rich type. These data indicate that higher levels of G proteins are present on bat cell surfaces than are found on surfaces of other animal species cells, perhaps explaining in part the decreased M74-G fusion activity of G proteins of this African henipavirus. Additionally, coexpression of F and G in HeV and NiV typically results in the formation of multinucleated giant cells, while the syncytia produced by F and B glycoproteins of the M74 henipavirus produce smaller syncytia in nonbat cells.

The proteolytic activation of M74 F proteins is also slower than that found in NiV. The goal of the replication strategies of M74 may therefore not be the formation of high numbers of progeny virions, but rather the production of a persistent infection (Krüger *et al*. 2014).

F glycoprotein activation requires endocytosis via clathrin-mediated coated pits, cleavage by host cell endosomal cathepsin L or B, and recycling to the cell surface (reviewed by Weis *et al*. 2014). The *Pteropus alecto* and *Rousettus aegyptiacus* cathepsin L proteases are highly conserved compared with those of other mammals and cleave HeV F protein with similar kinetics (El Najjar *et al*. 2015). Most paramyxoviruses, however, including measles and parainfluenza virus 5, use the furin protease, located within the Golgi network to cleave and activate the F protein. The furins of *P. alecto* and *R. aegyptiacus* cell lines are also highly conserved with other mammalian furins and catalytically activate paramyxovirus F proteins. The C-terminus of *P. alecto* furin, however, has significant amino acid variation in comparison with other furins and process parainfluenza virus 5's F protein more rapidly than in other mammalian species. Bat-specific differences appear to exist in the cellular localization of furin and these may influence its accessibility to the F protein of many paramyxoviruses (El Najjar *et al*. 2015). The furin in a *P. alecto* kidney cell line had higher activity than those from other bat cell types and might influence viral localization with the bat host. Interestingly, furin activity in a bat lung cell line was significantly lower and slower than activity in the two human lung cell lines (Nahar *et al*. 2015).

The GH-M74a henipavirus G glycoprotein can bind to its primary henipavirus receptor, ephrinB2, in a *H. monstrosus*-derived cell line that is permissive to NiV, however, the GH-M74a F glycoprotein was only able to induce limited syncytia formation in these cells and none in Vero African green monkey kidney cells or in an *E. helvum* kidney cell line (Weis *et al*. 2014). Its cleavage is delayed, resulting in reduced expression of fusion-active GH-M74a F protein and inhibited GH-M74a infection of *H. monstrosus* cells due to impaired trafficking and cell surface expression (Weis *et al*. 2014). While NiV shares 80–90% nucleotide identity with HeV in the F and G envelope glycoproteins, GH-M74a henipa-like viruses have only 70% and 40% nucleotide homology and 56 and 26% identity with NiV and HeV F and G glycoprotein genes, respectively (Pernet *et al*. 2014). The GH-M74a henipavirus also has a structurally distinctive receptor-binding scaffold and its G glycoprotein is antigenically distant from those from Asiatic NiV and HeV (Lee *et al*. 2015). Mapping the GH-M74a G glycoprotein sequence onto the crystal structure of NiV-glycoprotein bound to ephrinB2 revealed that most of the sequence conservation between these two viruses occurred at the receptor-binding interface region of the bat glycoprotein (Pernet *et al*. 2014). Both of these viruses as well as HeV use human ephrinB2 as their primary host cell-surface receptor. NiV, however, also contains a secondary ephrinB2 interaction site, allowing for more efficient receptor-mediated entry when compared with GHV-M74a. Additionally, GH-M74a is not able to bind to ephrinB3 (Lee *et al*. 2015). Together, the evidence of conserved receptor usage suggests GH-M74a is indeed a member of the henipavirus genus in Africa, even if it is only distantly related to NiV and HeV (Pernet *et al*. 2014). This also suggests the possibility that GH-M74a might be able to infect humans and may potentially be the causative agent of misdiagnosed malaria-associated encephalitis or other fevers of unknown origin (Lee *et al*. 2015). There is, however, currently no evidence to support that such zoonotic spillover has occurred.

3.4 HENDRA VIRUS

3.4.1 Hendra virus in Australian bats, horses, and humans

HeV was first detected in Australia in 1994. Antibody-positive members of all four species of mainland Australian flying foxes (*P. alecto, Pteropus poliocephalus, Pteropus scapulatus*, and *Pteropus conspicillatus*) have been reported in Australia. Several other bats have also been found to be seropositive: *Dobsonia moluccense, Dobsonia andersoni, Pteropus neohibernicus, Pteropus capistratus, Pteropus hypomelanus*, and *Pteropus admiralitatum* from the north coast of Papua New Guinea and other areas in the region (reviewed by Mackenzie 1999). Due to habitat alterations, flying foxes are increasingly moving into urban environments, thus increasing their contact with humans. Flying foxes are believed to serve as HeV's primary reservoir, from which it has spilled over into horses at least 51 times as of 2015 and, from them, into humans on at least seven occasions (reviewed by Goldspink *et al*. 2015). While the route of the hypothesized interspecies transmission of HeV from horses to bats has yet to be established, horses are known to shed HeV from nasopharyngeal secretions, urine, feces, and blood (reviewed by Middleton & Weingartl 2012). Even though antibodies against HeV have been found in all of the above Australian flying fox species throughout Australia, only the tropical and subtropical regions of the eastern part of the continent have reported HeV disease in horses or humans, with no disease in the southern, temperate part of Australia (Burroughs *et al*. 2016).

Field *et al*. (2015) performed a spatiotemporal analysis of HeV in Queensland and New South Wales, Australia. Pooled urine samples ($n = 13\,968$) were collected monthly over a 3-year period from under 27 roosts of the four species of flying foxes present in the country. A nonlinear relationship was found to exist between mean HeV excretion prevalence and five latitudinal regions. Analysis of HeV RNA indicated that viral excretion is moderate in northern and central Queensland; highest in southern Queensland and northern New South Wales, especially during the winter; moderate in central New South Wales; and negligible in southern New South Wales. HeV RNA was detected in areas containing both *P. alecto* or *P. conspicillatus*, but was absent or very low in roosts containing only *P. poliocephalus*. Since extreme periodic increases in numbers of *P. scapulatus* in some roosts were not associated with increased HeV detection in horses, they do not appear to be a significant source of zoonotic spillover in eastern Australia (Field *et al*. 2015).

A later spatial study in southern Australia concurred with the above findings and implicated that only *P. alecto* and *P. conspicillatus* are likely candidates for indirect transmission to humans via horse intermediates. These two species of flying fox have a tropical and subtropical distribution. RNA from pooled urine samples from a *P. poliocephalis* colony were tested several times per month for 26 months for the evidence of paramyxovirus. No HeV RNA was found in 872 *P. poliocephalis* samples, however RNA from four other paramyxoviruses was present: Yara Bend paramyxovirus in 1.9% of the bats and Geelong paramyxovirus, Teviot virus, and Cedar virus in less than 1% of the bats (Burroughs *et al*. 2016). Cedar virus is a nonpathogenic henipavirus related to HeV. Cedar virus was also isolated from the bat urine pools. Antibodies to HeV were present in 14.6–44.5% of the tested bats (dependent upon the parameters of seropositivity used in the studies), and was typically greater in juveniles. Antibodies against

Cedar virus were found in 21.2–51.1% of tested bats. These findings support the contention that *P. poliocephalis* is not a primary reservoir for HeV spillover into horses (Burroughs *et al*. 2016).

HeV has been detected in lung, spleen, liver, kidney, and heart and vascular tissue of experimentally infected *P. alecto* or *P. poliocephalis* and naturally infected flying foxes' uterine fluid and pooled fetal lung and liver tissue from aborted *P. poliocephalis* and *P. alecto* fetuses (reviewed by Goldspink *et al*. 2015). The rate of seropositivity increases during late-stage gestation and early lactation, as does the temporal association with spillover events, rather than during the birthing period (reviewed by Goldspink *et al*. 2015). A separate study examined the likelihood of various routes of viral transmission from naturally infected wild flying foxes in Australia ($n = 1410$). HeV nucleotides were detected in at least one sample (3.0%) of *P. alecto*. The prevalence and amount of viral RNA was highest in urine or urogenital samples (4% and 2%, respectively), but RNA was also found in serum (1%), rectal (2%), nasal (1%), and oral (1%) samples, implicating urine as the most important source of HeV transmission. All detections of viral RNA from female *P. alecto* clustered in their period of early to mid-gestation (Edson *et al*. 2015b). Interestingly, no HeV RNA was found in similar samples from *P. poliocephalus* ($n = 1168$), suggesting that the latter species of flying foxes are less important as HeV reservoirs (Edson *et al*. 2015b). Interestingly, maternal immunity has been reported to wane faster in *P. scapulatus* than in other flying fox species.

3.4.2 Factors affecting levels of Hendra viruses in bats and the potential for zoonotic transmission

In the Northern Territory of Australia, increases in seroprevalance in *P. scapulatus* occur when the bats are undergoing nutritional stress. This suggests that alteration of flying fox food sources due to factors such as habitat loss and climate change might affect HeV infection and transmission in this flying fox species (Plowright *et al*. 2008). Some factors that affect food availability and nutritional stress include anthropogenic habitat loss, habitat alteration, roost disturbance, and bat urbanization. Since this bat does not appear to be an important HeV reservoir in eastern Australia (discussed below), similar studies of *P. alecto* and *P. conspicillatus* from that region would be of value.

The trend of increasing urban habituation among flying foxes may escalate their contact with human and domestic animal populations. Disease modeling also suggests that urbanization decreases bat migratory behavior and may lower bat population immunity, thus producing more intense outbreaks after viral reintroduction into a locale (Plowright *et al*. 2011). Most of the known HeV outbreaks occurred in the vicinity of urbanized or sedentary groups of flying fox. When waning maternal immunity is included into the models, peak prevalence coincides with peak risk of HeV annual zoonotic spillover. The models also suggest that alteration of flying fox ecology due to human activity may lead to less frequent but more intensive lethal outbreaks of HeV in human and horse populations (Plowright *et al*. 2011). A later study by the same group of researchers examined the effects of increased urbanization of flying foxes upon their levels of cortisone, a major stress and immunosuppressive hormone, which may increase HeV excretion. The differences in mean urinary cortisol concentrations before, during, and after roost disturbance (22.7, 27.2, and 18.4 ng/ml, respectively) as well as the mean

HeV prevalence (4.9, 4.7, and 3.4%, respectively) were, however, not significant ($p=0.440$) (Edson *et al*. 2015a).

Goldspink *et al*. (2015) found HeV RNA in 6.2% of spleen, kidney, liver, lung, or blood of archived, naturally infected flying foxes ($n=295$), particularly *P. alecto* and *P. conspicillatus*, but not *P. scapulatus* and in only 1% of *P. poliocephalus*. The spleen had the highest rate of viral RNA detection and may play an important role in infection maintenance, in processing of viral components for the immune response, or in recrudescence. Importantly, no HeV RNA was found in the placenta or in fetal tissues, so vertical transmission appears to play, at most, a minor role in intraspecies or interspecies transmission among bats or to horses, respectively. Also, the presence of HeV RNA in the kidney suggests that initial mucosal replication occurs following entry via the oronasal route, followed by systemic infection and transit to the kidneys, with urine being a key factor in transmission to horses (Goldspink *et al*. 2015).

A study of factors underlying seropositivity of *P. conspicillatus* was conducted in a single colony in northern Australia close to the site of an outbreak of HeV infection in horses and humans in late 2004. The presence of neutralizing antibody in blood samples in these bats ($n=521$) was examined for 25 months in six sampling sessions (Breed *et al*. 2011).Unlike the acute and self-limiting episodic infection pattern characteristic of measles and rinderpest viruses, seroprevalance gradually increased during the study, suggesting an endemic infection of this species of flying foxes (Breed *et al*. 2011). Age of the bats, pregnancy, and lactation were significant risk factors for producing neutralizing antibodies. The titers were significantly higher in females than males, especially in pregnant animals due to their altered immune status. Early during lactation, 75% of female bats with pups were seropositive. Temporal variation in titers and viral RNA in bat urine indicates that herd immunity may vary on a seasonal basis. An effective anti-HeV equine vaccine is available, however, it has not been often used. The increase in HeV RNA secretion by bats in winter months suggests the utility of a program to promote equine HeV vaccination in autumn in order to decrease the number of susceptible horses and spill-over into humans during the high-risk winter period of bat excretion in the affected regions of tropical and subtropical Queensland and New South Wales (Field *et al*. 2015).

3.5 NIPAH VIRUS

A phylogenetic study of the evolution of NiV indicates that the root of the tree originated in 1947, during which time the virus entered Southeast Asia. NiV separated into two main clades (I and II), with the introduction of clade I (NiV-B) in 1995, involving Bangladesh, Thailand and India, and the introduction of clade 2 (NiV-M) in 1985, involving primarily Cambodia and Thailand. Trading of infected pigs and the long-distance migration of *Pteropus* bats may have been at least partially responsible for NiV spread (Lo Presti *et al*. 2016).

3.5.1 Nipah virus in humans and pigs

NiV is known to infect a wide range of cell types, including parenchymal cells, endothelial cells, smooth muscle cells, neurons, monocytes, and dendritic cells (Gupta *et al*. 2013).

Infection of humans with NiV, like HeV, results in severe respiratory and neurological disease and has a fatality rate of 40–90%. Malaysia, India, Bangladesh, and Singapore have experienced outbreaks of Nipah virus infection of pigs and humans, leading to the death of more than 240 people. Human deaths have been reported in Bangladesh nearly every year since 2001 and, in India, in 2001 and 2007 (Sendow *et al*. 2013). Since pigs may serve as an amplifier host, over 1 million pigs were culled, further damaging the weak economies of the region. NiV infection in pigs generally has higher transmission rates, but lower morbidity and mortality than is seen in horses infected with HeV. During an outbreak in pigs, infection rate approached 100%, however, most of pigs did not display clinical disease and the mortality rate was only 1–5%. Those pigs which did develop neurological disease displayed muscular spasms, rear leg weakness, uncoordinated gate, agitation, tetanus-like spasms or seizures, inability to swallow, and frothy salivation. Animals which developed respiratory illness had high fever, increased respiratory rate or forced respiration, and a harsh nonproductive cough. Pig-to-pig transmission might be via the airborne route since NiV has been located in upper and lower respiratory tract epithelium and in the lumen of airways. Rarely, horses, goats, cats, and dogs may be naturally infected with NiV (reviewed by Middleton & Weingartl 2012).

3.5.2 Nipah virus in bats from Malaysia and Indonesia

The initial NiV outbreak in Malaysia in 1998 alone resulted in 265 human encephalitis cases (reviewed by Breed *et al*. 2011). This outbreak in humans was preceded by a large outbreak in local pigs and contact with sick pigs was a major risk factor for human illness. NiV was isolated from the cerebrospinal fluid of a patient in 1999. Two area fruit bat species (*P. hypomelanus* and *P. vampyrus*) were found to produce neutralizing antibodies to NiV and the virus was also isolated from *P. hypomelanus* urine and from swabs of their partially eaten fruits (Chua *et al*. 2002; Johara *et al*. 2001). Molecular sequencing from the nucleocapsid gene to the end of the glycoprotein gene (encompassing the major structural genes and the immunosuppressive P gene) confirmed its identity as Nipah viruses that contain a sequence deviation of five to six nucleotides from a human isolate. The presence of neutralizing antibodies and isolation of NiV from a flying fox implicates this group of bats as natural NiV reservoirs (Chua *et al*. 2002). It was postulated that pigs are infected by ingesting partially eaten fruit that contained infectious bat saliva. In a study conducted in 15 sites throughout Thailand from 2002 to 2004, NiV IgG antibodies were detected in 6.3% of tested bats (15.4% of *P. hypomelanus,* $n=26$; 2.6% of *P. vampyrus,* $n=39$; 9.3% of *P. lylei,* $n=318$; and 1.3% of *H. larvatus,* $n=74$), but no human infections were reported in that country. Viral RNA was detected in samples of pooled urine from the frugivorous *P. lylei*, as well as saliva from *P. lylei* and the insectivorous *Hipposideros larvatus* (Wacharapluesadee *et al*. 2005).

A 2002 study of *P. vampyrus* from two Indonesian islands (Sumatra and Java) found neutralizing antibodies attributed to NiV in 35.7% of the bats and a prevalence of 2.9% attributed to HeV. Many of the antibodies reacted to both henipaviruses, but had a higher titer to NiV (Sendow *et al*. 2006). A later study in *P. vampyrus* in Sumatra detected NiV RNA is 9% of pooled urine samples ($n=22$), 4% of bladder tissue ($n=27$), and in 2% of oropharangyeal swabs ($n=47$) (Sendow *et al*. 2013). The RNA sequences in Sumatra were very similar to those found in Malaysia, which is to be expected since flying foxes are known to travel between peninsular Malaysia and Sumatra over a sea

distance of less than 50 km. RNA from a novel paramyxovirus was also detected in *Acerodon celebensis* and *P. vampyrus* bats in Indonesia (Sasaki *et al*. 2012).

3.5.3 Nipah virus in bats from India and Bangladesh

Almost half of tested *Pteropus giganteus* ($n=41$) were seropositive for NiV in a 2008 study in northern India (Chua *et al*. 2002; Epstein *et al*. 2008). A later study also detected viral RNA in liver tissue of 3.2% of tested Indian *P. giganteus* ($n=31$) (Yadav *et al*. 2012).

Two strains of NiV (NiV-MY and NiV-BD) have been reported in *P. lylei* in bats. The Bangladesh strain from Bangladesh and India is predominantly associated with higher rates of respiratory disease. None of this strain's viral RNA could be detected in the bats, however (reviewed by Wacharapluesadee *et al*. 2016). A more recent study found NiV-MY RNA in 2.7% of the samples of *P. hypomelanus* urine ($n=184$) in southern Thailand. Molecular analysis determined the virus to be the same strain as that previously reported in Malaysia (Wacharapluesade *et al*. 2016). Human infection by NiV-MY occurs almost exclusively by direct transmission from infected pigs that harbor NiV in their lungs and airways. The overall fatality rate is 38.5%. Human infection with NiV-BD, however, results from bat-to-human and human-to-human transmission and has an overall fatality rate of 73% (reviewed by Clayton *et al*. 2013).

An endemic infection pattern can maintain viral presence using a much smaller critical host population size, as had been previously seen in *Pteropus lylei* in Thailand (Breed *et al*. 2011). RNA of both NiV-BD and NiV-MY was detected in urine of *P. lylei* in Central Thailand. The Bangladesh strain was dominant and was almost exclusively detected between April and June, while the Malaysian strain was found between December and June. These results do not entirely support the necessity of breeding activity in the spillover of NiV strains into humans and indicate that NiV strain-to-strain differences occur (Wacharapluesadee *et al*. 2010).

3.5.4 Interspecies Nipah virus transmission via date palm sap and bat urine

The factors associated with acquisition of NiV-associated encephalitis were examined during an outbreak in Bangladesh between December 15, 2004 and January 31, 2005. Of the 12 patients identified, 92% died. Two of the three available serum specimens contained IgM and IgG antibodies against NiV. The only factor that was found to be significantly associated with encephalitis was drinking raw date palm sap (64% in those infected versus 18% among normal controls). Sap is collected from mid-December to mid-February. Unfortunately, the investigators did not test the sap itself for virus since the outbreak had ended. *P. giganteus* fruit bats are well-known to contaminate raw sap with saliva by licking the date palm sap-producing surface and drinking from clay pots used to collect the sap at night. Additionally, bat excrements are often found in the sap. While there is some evidence of occasional NiV transmission from domestic animals, that pathway represents a much less important route of transmission in Bangladesh compared with date palm sap consumption, therefore implicating fruit bats as the primary route of human infections in this outbreak as well as the 2008 outbreak in villages separated by 44 km and a river, and an outbreak in early 2011 (Luby *et al*. 2006; Rahman *et al*. 2012; Chakraborty *et al*. 2016). A separate study reported that NiV was isolated

from urine and chewed and dropped fruit beneath free-living colonies of *P. hypomelanus* on Tioman Island. While animal or human ingestion of this date palm fruit was suggested as a means of transmission, it has yet to be associated with human NiV infection in Bangladesh (Khan *et al*. 2012).

A study conducted between 2010 and March 2014 of 14 people in Bangladesh who were hospitalized with NiV encephalitis implicated consumption of illegal fermented date palm sap in eight cases in which patients had not drunk fresh sap or had contact with sick animals, while six patients were care-givers to an infected person (Islam *et al*. 2016). Of note, during fresh date palm sap collection, harvesters clean and dry the collection pots between collections, however, during the production of fermented sap, the same earthen pot is used for collection for several days without cleaning in order to allow yeast to grow in the bottom of the earthen pot. The yeast helps to ferment the palm sap. Fermented sap typically sells for 2.5 times the price of fresh sap and is easier to produce (Isalm *et al*. 2016). Typically, enveloped viruses, like NiV, are susceptible to solutions of 60%–70% alcohol. In India, however, fermented sap only contains 5%–8% alcohol (reviewed by Islam *et al*. 2016). In addition to Bangladesh, fermented palm sap is harvested for fermentation in areas with fruit bats in Australia, Asia, and Africa.

Winter is the prime sap harvesting season and the ambient temperature is 15–28 °C. Risk factors identified during four outbreaks in Bangladesh during the winter collecting seasons from 2001 to 2004 include contact with a sick cow or pigs and climbing trees. Contact with ill persons or their secretions was also a major risk factor in three of these four outbreaks, stressing the importance of human-to-human transmission in amplifying infection in humans (Hsu *et al*. 2004; reviewed by Luby *et al*. 2006; Chakraborty *et al*. 2016). A separate study in Bangladesh also implicated human-to-human transmission as the major risk factor for NiV infection, since 91.7% of the patients ($n=36$) had close prior contact with another patient (Gurley *et al*. 2007). One patient in particular was associated with 22 of the other cases, suggesting the possibility of "super-spreaders," as seen during the SARS epidemic in China. NiV RNA was also detected on hospital surfaces and in human respiratory secretions. Hand washing was found to be protective (Gurley *et al*. 2007). Nosocomial transmission of NiV has been reported during a NiV outbreak in India, however, was not seen in other outbreaks in Malaysia and Singapore (reviewed by Gurley *et al*. 2007).

In Sub-Saharan Africa, several studies have reported that *E. helvum* Old World fruit bats which are hunted and eaten are seropositive for henipavirus antibodies. Additionally, henipa-like RNA is present in 1.4% of fecal samples ($n=215$) from the straw-colored fruit bat (*E. helvum*) in Ghana, although the feces might have been contaminated by bat urine (Drexler *et al*. 2009). Non-neutralizing as well as neutralizing antibodies to NiV and HeV were found in healthy *E. helvum* bats in Ghana (39% and 22%, respectively; $n=59$) as well as in 1% of *E. gambianus* (to NiV, but not to HeV). About 5% of sera from pigs that spent time under an *E. helvum* roost were found to be seropositive in a separate study in Ghana ($n=97$), however, no antibodies were found in a small sample of other domestic species (Hayman *et al*. 2008, 2011).

Henipaviruses are viable for short periods of time over a wide pH range, remaining viable for 1 h at pH 3–11 and pH 4–11 for NiV and HeV, respectively. These viruses are very sensitive, however, to desiccation and high temperatures. When desiccated, henipaviruses survive for less than 15 min at 37 °C. At an optimal temperature of 22 °C, desiccated HeV decreased by greater than 3 logs by 30 min and NiV decreased by greater

than 2 logs by 60 min. HeV incubated in *Pteropus* urine adjusted to pH 7 remained viable for at least 4 days at 22 °C, but less than 1 day at 37 °C. In urine at pH 2, survival time was greatly diminished at both temperatures. They are also able to survive for at least a week in palm sap (neutral pH) without a decrease in titers at a temperature of 22 °C. On mango flesh, survival time varied from hours to greater than 2 days, depending on temperature and fruit pH. Viruses persisted for more than 3 days in lychee juice, far longer than in Pawpaw or mango juice, with pH playing a major role (Fogarty *et al*. 2008; de Wit *et al*. 2014). Seasonal or migratory-induced changes in diet of fruit bats may affect urine pH and viral survival, in turn affecting seasonal interspecies transmission. Additionally, the urinary pH of *P. alecto* and *P. vampyrus* differs significantly despite being fed similar diets. This might affect the ability of various bat species to spread henipaviruses (Fogarty *et al*. 2008). A 2008 study in northern India found no significant difference in seroprevalance between sexes or between lactating and nonlactating bat females, suggesting that pregnancy and lactation do not affect NiV infection (Epstein *et al*. 2008).

3.6 CEDAR VIRUS

A 2012 report described the isolation and characterization of a novel henipavirus, Cedar virus, from the urine of a mixed colony of flying foxes in Australia (predominantly *P. alecto* and some *P. poliocephalus*) (Marsh *et al*. 2012). The virus's large genome size (over 18 000 nucleotides) and organization are very similar to those of the other two henipaviruses. Its nucleocapsid protein is also antigenically cross-reactivity with HeV and NiV and it uses the same host cell receptor. Cedar virus is able to replicate in ferrets and guinea pigs, as is the case with the other henipaviruses, and stimulates production of neutralizing antibodies in the absence of clinical disease. These antibodies are not cross-neutralizing with other henipaviruses (Marsh *et al*. 2012). Unlike almost all other paramyxoviruses, Cedar virus P gene does not undergo RNA editing, thus its V protein, critical to HeV and NiV evasion of the host innate immune system, is absent, as discussed below. Importantly, Cedar virus is nonpathogenic to humans.

3.7 PROTECTIVE BAT RESPONSES TO HENIPAVIRUS INFECTION

3.7.1 The interferon/STAT pathway and henipaviruses

The mammalian type I interferon (IFN) group of antiviral cytokines produced by the innate immune system consists of IFN-α and IFN-β. They are part of the IFN/STAT1/STAT2 (Signal Transducers and Activators of Transcription) pathway and its associated IFN-stimulated genes. Viral infection of host cells generally induces this pathway, during which STAT1 and STAT2 proteins undergo nuclear localization, then activate expression of several antiviral genes. Bat type I IFN and STAT genes function in a similar fashion to those of humans (reviewed by Virtue *et al*. 2011a).

Most paramyxoviruses decrease IFN antiviral responses by directly inhibiting STAT protein activity. The mechanisms vary within viral families and among species in the same genus, ranging from cytoplasmic sequestration by mumps virus to polyubiquitylation and

proteasomal degradation of STAT1 and STAT2 by human parainfluenza virus type 2. The diverse means of inhibiting STAT activity usually involve a conserved immunosuppressive viral "V" protein (reviewed by Rodriguez *et al*. 2003).

Pathogenic HeV and NiV diminish the host's innate immune response, especially the interferon type 1/JAK/STAT pathway and its many downstream interferon-induced gene products which are necessary for robust anti-viral immunity (Virtue *et al*. 2011a). Henipavirus *P* gene products are responsible for at least some of the IFN blockage. In many paramyxoviruses, the *P* gene produces four proteins, the P, V, W, and C proteins, produced by RNA editing of the *P* gene (reviewed by Shaw 2009). The editing process results from the viral polymerase stuttering at a run of A and G residues, leading to the addition of nontemplated G residues into the nascent mRNA chain. In its unedited form, P is produced, which acts as a cofactor for the polymerase during viral RNA synthesis. The V protein results from a frameshift due to insertion of an extra, nontemplated G residue, while the W protein is produced by a further shift in reading frames by the addition of a second G nucleotide. NiV and HeV edit their *P* genes at a very high frequency, leading to the addition of up to 14 G nucleotides. Due to this editing process, P, V, and W proteins have a common N-terminal domain with 81% identity in the first 140 amino acids, but unique C-terminal domains (Rodriguez *et al*. 2003). The N-terminal domain of these three henipavirus proteins is 100–200 amino acids longer than that of morbilliviruses and rubulaviruses. The C-terminal of the henipavirus W protein is also longer than that seen in morbilli- and respiroviruses (reviewed by Shaw 2009). The C protein is produced from an alternative open reading frame within the transcripts of P, V, and W. It, like the P, V, and W proteins, inhibits cellular antiviral responses. The C protein inhibits proinflammatory cytokine activity by an unknown mechanism.

The henipavirus V protein blocks IFN-α, -β, and -γ signal transduction by sequestering STAT1 and STAT2, but not STAT3, in the cytoplasm by binding and trapping them in high-molecular weight complexes of approximately 500 kDa (Rodriguez *et al*. 2002, 2003). W protein also targets STAT1. The V and C proteins from human Morbilliviruses, Respiroviruses, Rubulaviruses, and Avulaviruses also suppress STAT1 activation, IFN-β transcription, or IFN-β production (Basler 2012).

In addition to blocking STAT nuclear localization, V and W proteins block production of IFN-β. Several cellular membrane viral recognition systems which utilize Toll-like receptor 3 (TLR) and RIG-I-like receptors (RLR), including MDA-5 and RIG-1, detect the presence of viruses and activate production of IFN-β cytokine. The V protein interacts with the cellular viral sensor protein MDA-5 via the V protein's cysteine-rich C-terminal domains to block MDA-5-mediated signaling and activation of IFN-β, while the W protein inhibits signaling via TLR3 and RLR (reviewed by Shaw 2009 and Basler 2012). Interestingly, the nonpathogenic Cedar virus does not produce V or W proteins and its P protein is less able to bind to or inhibit STAT1 nuclear accumulation or production of the interferon-inducible *MxA* gene (Lieu *et al*. 2015). Additionally, while both Cedar virus and HeV induce similar levels of IFN-α in the HeLa cell line, Cedar virus induces significantly higher levels of IFN-β (Marsh *et al*. 2012). Increased IFN-β activity may be crucial for host protection and may be responsible for the lack of Cedar virus pathogenicity.

Henipavirus infection of *P. alecto* cell lines blocks expression of type I IFNs, STAT1 and STAT2 activity, and expression of IFN-stimulated genes (Virtue *et al*. 2011a). These bat cell lines were derived from several different bat cell types taken from several organs (lungs, kidney, and fetuses), indicating that the blockage is not cell

type-specific (Virtue *et al*. 2011a). Additionally, exogenous IFN alone did not restore the IFN signaling response in henipavirus-infected bat cells. While type I IFN production is blocked by large levels of viral protein in henipavirus-infected human cells, the IFN signaling pathway itself is still functional (Virtue *et al*. 2011b).

Mammalian type III IFNs (IL-28B and IL-29) have anti-viral activity as well. Their production is also suppressed in henipavirus-infected bat cells, unlike the upregulation of type III interferons in bat cells infected by Tioman virus, another bat paramyxovirus (Zhou *et al*. 2011).

3.7.2 Antibodies and henipaviruses

Serological studies of anti-henipavirus antibodies in pteropid bat colonies have found seroprevalence as high as 59%, in contrast to viral isolation and molecular studies, which may be found in only 1% of the bats (reviewed by Epstein 2013). Nevertheless, many studies of viral prevalence and length of infection rely primarily or solely upon detection and levels of IgM and IgG or, more reliably, on the presence of neutralizing antibodies. While detection of neutralizing antibodies is the gold standard of the serological methods, it requires the use of very rare BSL4 facilities due to the great risk in working with live henipaviruses, thus restricting its usage.

Studies of seroprevalance safely provide important information, especially in juvenile animals that lack cell-mediated immunity, including antibody production. During this period of their lives, elements of the innate immune system together with maternal IgG which had been passed through the placenta during fetal development, provide protection against microbial threats. Unlike the case in humans, IgG is also found in bat mother's milk. This protection decreases as the transferred maternal antibodies become nonfunctional over a period of months. Seasonal birth of pups increases the populations' overall susceptibility to microbes once maternal immunity wanes. A study of antibodies against HeV in naturally infected, pregnant *P. alecto* bats and their offspring found that HeV-specific antibodies were transferred from dam to pup. Levels of antibodies decreased over a period of 255 days, with a mean terminal phase half-life of 52 days (Epstein *et al*. 2013). The transferred maternal immunity lasted from 7.5 to 8.5 months, which is slightly longer than that of humans. Similar kinetics of maternal antibody-mediated protection were reported in captive *P. hypomelanus* vaccinated with canine distemper virus antigen, except for a longer mean terminal phase half-life of 96 days (Epstein *et al*. 2013).

3.7.3 Apoptosis

Infection of a bat kidney cell line with HeV led to NF-κB activation and upregulation of the extrinsic apoptosis pathway via the tumor necrosis factor-related apoptosis inducing ligand (TRAIL). HeV also sensitized bat cells to TRAIL-mediated apoptosis by upregulating their death receptor gene expression. Upregulation of anti-apoptotic elements was also seen in HeV-infected bat kidney cells. Nevertheless, bat cells significantly increased their rate of apoptotic cell death by 48–72 h post-infection (Wynne *et al*. 2014). Experimental infection of a human kidney cell line with HeV, by contrast, resulted in either downregulation of proapoptotic proteins or upregulation of anti-apoptotic

proteins and no subsequent increase in apoptotic death of infected cells. A high level of rapid (within 24 h) syncytia formation and cell death by cytopathic effect did, however, occur in HeV-infected human, but not bat, cells. The HeV F protein is responsible for cell fusion and syncytia formation and higher levels of F gene expression are seen in human versus bat cells (Wynne *et al*. 2014). *In vitro*, therefore, HeV-infected bat kidney cells are eliminated by apoptosis after several days, while the infected human kidney cells died more rapidly by a different mechanism.

The strength of apoptosis induction by HeV is not equal in all bat or human cell lines. A bat fetal cell line developed a strong proapoptotic response via caspases 3 and 7, while bat brain and lung cell lines produced less of a response. In contrast to the human kidney cell line discussed above, a strong caspases 3 and 7 response occurred in two other human cell lines, indicating that HeV-induced apoptosis is not specific to bat cells (Wynne *et al*. 2014). It is noteworthy that the apoptotic response in bats following *in vivo* HeV infection differs from the *in vitro* response. TUNEL staining of tissue sections showed no increase in the proportion of apoptotic cells from bat kidney or spleen following *in vivo* infection (Wynne *et al*. 2014).

NiV has different effects upon apoptosis in human cells. It infects dendritic cells and replicates within them to a low level. NiV activates and induces expression of antiviral, proinflammatory TNF-α, IL-1α, and IL-1β cytokines and the IL-8 and IP-10 chemokines, which attract neutrophils and activated T cells and natural killer cells into the area, respectively. Expression of costimulatory CD40, CD80, and CD86 molecules is additionally upregulated in humans. While these actions would normally aid in T lymphocyte activation, NiV also decreases dendritic cell expression of the MHC class II molecules required for initiation of T helper cell responses. NiV also decreases levels of anti-apoptotic bcl2 and increases levels of the active form of proapoptotic caspase 3, leading to dendritic cell activation-induced cell death. Infected dendritic cells partially and inadequately activate T cells, resulting in apoptosis rather than stimulation (Gupta *et al*. 2013). Another immunosuppressive paramyxovirus, measles virus, alters human dendritic cell function, leading to T cell anergy.

3.8 METHODS OF PREVENTING HENIPAVIRUS INFECTION

A 2010 study examined the usefulness of several intervention devices designed to prevent bats from contaminating date palm sap (Khan *et al*. 2012). This study employed four types of materials to construct skirts for intervention, each used on 15 trees: bamboo, material from the local dhoincha plant, jute stick, and polythene. The skirts covered the shaved section of the tree, sap stream, tap, and collection pot. Sixty trees without skirts served as negative controls. Motion sensor activated infrared cameras detected bat contact with sap. Bats contacted sap in only 2% of trees with skirts but in 83% of control trees. No bats contacted sap in trees with bamboo, dhoincha skirts, or polythene covering. Contact did occur one night in sap covered with a jute stick skirt. Importantly, trees with skirts produced similar amounts of sap as control trees with no change in sap clarity or turbidity (Khan *et al*. 2012).

The usage of skirts is particularly important in light of the lack of success of public education concerning NiV and raw sap consumption. In a survey of villages in Bangladesh, nearly 50% of the people drank raw sap during the previous season, with at

least 37% drinking it at least once a month (Nahar *et al*. 2015). Only 5% of those surveyed knew about NiV, but 37% of the people had heard of a disease transmitted through raw sap consumption. Those who knew of such a disease, however, were as likely to drink raw sap as those who did not know of a disease connection (Nahar *et al*. 2015). One factor that impedes education efforts is the lack of access to mass media often occurring in producers of fermented sap due to their ethnic, religious, and linguistic minority status in Bangladesh (Islam *et al*. 2016).

Other measures to prevent zoonotic transmission of henipavirus include changing agricultural practices by placing buffers between fruiting trees and domestic animals, especially horses and pigs (Smith & Wang 2013). Passive surveillance of dead animals may warn of potential threats of zoonotic spread. A vaccine against HeV was released in 2012 that may reduce spread of this virus between horses and from horses to humans in areas of high risk. Outbreak management plans could include wearing appropriate protective equipment when caring for patients and animals or restricting sale of known viral animal hosts. Advanced planning of public health measures is necessary and might utilize enhanced surveillance and infection control in horses, pigs, and humans. Quarantine and contact tracing decrease viral transmission during outbreaks as well.

3.9 RUBULAVIRUSES

3.9.1 Bat parainfluenza virus

Bat parainfluenza virus was isolated from pooled organs of *Rousettus leschenaultia* ($n = 70$) in a study of viruses in bats residing near a stud farm in India (Pavri *et al*. 1971). This virus is similar to, but distinct from, human parainfluenza virus-2 (Hollinger & Pavri 1971). Neutralizing antibodies against the virus were present in 7.1% of local tested bats. The farm contained orchards growing citrus, guava, and mango. Horses were raised at the farm as well as cows, buffaloes, and deer. This virus caused syncytia formation and cytopathic effect in cell culture and was lethal for inoculated infant mice. Antibodies to bat parainfluenza virus were also present in 10% of tested local human serum samples ($n = 200$). Human disease was not mentioned in this report, nor was isolation of this bat virus from humans.

3.9.2 Menangle virus in bats and domestic animals

Menangle virus was first isolated in 1997 in New South Wales, Australia, from lung, brain, and heart tissue of stillborn piglets with deformities that included extensive degeneration and necrosis of both gray and white matter of the central nervous system together with infiltration of inflammatory immune cells. This paramyxovirus is associated with decreased pregnancy rate and litter size and increased prevalence of mummified fetuses. Seropositive animals were found in three piggeries, in at least two humans exposed to these pigs, and in area fruit bats (Philbey *et al*. 1998). In the piggery from which the virus was first found, over 90% of the serum from pigs of all ages ($n = 88$) contained high titers of neutralizing antibodies from May to September 1997, but none were present in sera before that time. Neutralizing antibody was found in 32.9% of *P. poliocephalis* ($n = 79$), 55% of *P. alecto* ($n = 20$), and 40% of

P. conspicillatus ($n = 10$), but not in *P. scapulatus* ($n = 15$). Some of the positive samples were collected in 1996, prior to known infections in pigs. Other local animals, including rodents ($n = 19$), birds ($n = 13$), cattle ($n = 60$), sheep ($n = 70$), cats ($n = 25$), and a dog, were seronegative (Philbey *et al*. 1998). Menangle virus causes only a febrile illness with rash in humans.

3.9.3 Tioman virus in bats and humans

Tioman virus was isolated and partially sequenced from the urine of *P. hypomelanus* on an island lying off the coast of peninsular Malaysia (Chua *et al*. 2001). Only Menangle virus from Australia showed any serological cross-reaction with this paramyxovirus. Tioman virus could not be amplified using Menangle virus primers, however, indicating that they are two separate viruses. Approximately 3.0% of the humans of Tioman Island were found to be seropositive for Tioman virus ($n = 169$) and 1.8% were found to have neutralizing antibodies (Yaiw *et al*. 2007). On this island, it is common for residents to consume fruit partially eaten by bats (19% of tested residents). Fortunately, Tioman virus infection has not been linked to any pathology.

3.9.4 Tuhoko viruses in bats

RNA from three novel rubulaviruses, Tuhoko virus 1, 2 and 3, were detected in 4.5% of the alimentary or respiratory samples from healthy Leschenault's rousettes (*R. leschenaultia*) in China (Lau *et al*. 2010). One third of the Tuhoko virus-infected bats were co-infected by bat-coronavirus HKU9. Although the Tuhoko viruses cluster with Menangle and Tioman viruses, their nucleotide sequences were less than 76% identical to other known rubulaviruses. Antibodies to Tuhoko viruses were found in 52–65% of tested *R. leschenaultia* sera (Lau *et al*. 2010). No pathology has been associated with infection.

3.9.5 Achimota viruses in bats

Two novel rubulaviruses, Achimota virus 1 and Achimota virus 2, were isolated from the urine of *E. helvum*. After sequencing, phylogenetic analysis revealed that Achimota viruses were rubulaviruses that clustered with Menangle, Tioman, and Tuhoko bat viruses. They appear to be related to many other rubulavirus fragments from a wide range of bat species throughout Africa, but have the highest amino acid identity with proteins from other fruit bat-derived rubulaviruses (Baker *et al*. 2013). Achimota viruses persist within fruit bat populations and appear to spread by horizontal transmission. Serological analysis indicates wide-spread exposure of *E. helvum* to Achimota viruses and, additionally, that human exposure to Achimota virus 2 may be occurring in Ghana and Tanzania. In humans, though, the titer was very low (20) in the three people who produced neutralizing antibodies ($n = 442$) (Baker *et al*. 2013). Seroprevalence among *E. helvum* from Africa in 2010 ($n = 126$) was 12–14% and 7–8% for Achimota virus 1 and Achimota virus 2, respectively. Prevalence of Achimota virus 1 was significantly higher in juvenile and adult bats than in sexually immature animals (Baker *et al*. 2013).

3.9.6 Sosuga virus in bats and humans

Sosuga virus is a member of the *Rubulavirus* genus from eastern Africa that is linked to a disease characterized by high fever, headache, generalized myalgia and arthralgia, stiffness in the neck, sore throat, maculopapular rash, and oropharynx ulcerations in a researcher collecting bats and rodents (Albariño *et al*. 2014). Sosuga RNA was isolated and sequenced from spleen tissue from 2.5% of *R. aegyptiacus* from the area ($n = 122$) (Amman *et al*. 2015). This virus is most closely related to Tuhoko virus 3 from *R. leschenaultii* fruit bats in southern China, but has only 57.4–84.0% amino acid identity with Tuhoko virus 3 proteins (Albariño *et al*. 2014). No evidence of this virus was found in other area bats, including 262 Ethiopian epauletted fruit bats (*Epomophorus labiatus*). Area rodents were not tested, so might serve as Sosuga viral vectors or reservoir hosts.

3.9.7 *Jeilongvirus* in bats

A study of various organs from deceased European insectivorous bats discovered a novel *Rubulavirus*. Subclinical pathology was found in the bats' kidneys, as well as in kidneys infected by two other novel paramyxoviruses discovered in this study (Kurth *et al*. 2012). The latter two viruses bore phylogenetic relation to the proposed paramyxovirus genus *Jeilongvirus*. They were found in the kidneys of the whiskered bat (*M. mystacinus*) and from pooled organs of the common pipistrelle (*Pipistrellus pipistrellus*). The other novel *Rubulavirus* was found in the lungs of a noctule bat (*Nyctalus noctula*) which had severe lung congestion (Kurth *et al*. 2012). The discovery of these three paramyxoviruses increased the known geographical location (Europe) and bat host type (insectivorous bats), suggesting that other, similar paramyxoviruses might be found in bats as well.

3.9.8 Mumps-like bat virus

The genome of a mumps-like bat virus from *Epomophorus minimus* has sequence homologies of about 90% in most genes to their counterparts in the human mumps virus. Bat and human mumps viruses appear to have a close antigenic relationship since polyclonal antibodies from bats cross-react with human mumps virus proteins (Drexler *et al*. 2012). The surface fusion and hemagglutinin glycoproteins of human and bat mumps viruses are also functionally related as demonstrated by the ability of either glycoprotein from the bat virus to cooperate with its human counterpart to induce syncytium formation. This is unusual among paramyxoviruses, in which the fusion protein usually requires a hemagglutinin from the same species for fusion to occur (Krüger *et al*. 2015). Human infection with mumps-like bat virus has not been reported at this time.

3.9.9 Mapuera virus in bats

Mapuera virus, another bat rubulavirus, was isolated from the salivary glands of *Sturnira lilium* fruit bats from Brazil (Zeller *et al*. 1989). It appears to be nonpathogenic to humans.

3.10 MORBILLIVIRUSES IN BATS

A study in Madagascar tested 947 bats from 52 capture sites from different bioclimatic zones, with different vegetation types and highly endemic biotic communities, for the presence of paramyxoviruses. Viral RNA was present in 10.5% of tested bats from six of seven families and in 16 of 31 bat species: 50.0% of *Triaenops menamena* were positive ($n=42$), 33.3% of *Coleura kibomalandy* ($n=6$), 18.2% in *Miniopterus gleni* ($n=22$), 17.9% of *Otomops madagascariensis* ($n=39$), 16.2% of *Mops leucostigma* ($n=68$), 15.5% of *Miniopterus griveaudi* ($n=116$), 15.0% of *P. rufus* ($n=20$), 10.4% of *Myotis goudoti* ($n=48$), 9.1% of *Miniopterus sororculus* ($n=22$), 9.1% of *Pipistrellus hesperidus* ($n=11$), 7.9% of *Mormopterus jugularis* ($n=152$), 7.1% of *Paratriaenops furculus* ($n=14$), 6.4% of *Chaerephon leucogaster* ($n=94$), 5.3% of *Miniopterus* cf. *ambohitrensis* ($n=19$), 5.3% of *Mops midas* ($n=19$), and 4.5% of *Miniopterus mahafaliensis* ($n=89$) (Mélade *et al*. 2016). Interestingly, all of the paramyxoviruses found in Malagasy bats are Mobillivirus-related viruses with little host specificity. The non-native black rat, *Rattus rattus*, is a significant reservoir of Morbillivirus-related viruses and may, therefore, be a major factor in the establishment of epidemiological interspecies bridges (Mélade *et al*. 2016).

Host switching is the major macro-evolutionary mechanism among viruses of Malagasy bats (Mélade *et al*. 2016). Host-switching involves the creation of a new host–parasite combination as a result of a parasite shifting to a new host, followed by specialization due to selection pressures, such as climate, season, and migration. It is the usual macro-evolutionary mechanism employed by RNA viruses. Paramyxovirus prevalence was 11.1% in insectivorous bats and only 3.8% in frugivorous bats. Viral prevalence differed greatly by province, altitude, and climate (4.5–15.2%). Mean prevalence rates at low, middle, and high elevation were 11.4, 8.9, and 3.5%, respectively. In humid, sub-humid, sub-arid, and dry zones, the mean infection rates were 5.4, 6.3, 12.0, and 10.9%, respectively, and 7.9 and 12.1% in bats captured during summer and winter seasons, respectively (Mélade *et al*. 2016). Mean annual temperature, but not mean annual rainfall, was found to have any overall relationship with infection in bats. Sites housing multiple bat species had higher infection rates compared with monospecific sites.

3.11 BELINGA BAT VIRUS

The RNA of Belinga bat virus, an unclassified paramyxovirus, was present in hearts and livers of 14.9% of African sheath-tailed bats (*Coleura afra*) from two caves in Gabon ($n=94$). One of these bats had diarrhea, severe hemorrhagic lesions in thoracic and abdominal organs, lung congestion, and pleurisy. The highest viral load is this bat was found in the heart. This virus is most closely related to two other unclassified paramyxoviruses of rodents, J virus and Beilong virus (65 and 66% nucleotide identity, respectively) (Maganga *et al*. 2014). Other bats sharing the caves and residing in close proximity to the infected bats were RNA-negative (*Hipposideros cf. ruber, Hipposideros gigas, Miniopterus inflatus, R. aegyptiacus*, and *Rhinolophus alcyone*) as were the cave's mosquitoes and bat flies.

3.12 LARGE, MULTIVIRAL STUDIES OF PARAMYXOVIRUSES IN BATS

3.12.1 Multiviral paramyxoviruses studies in Asia

A study of fecal material from 20 common frugivorous or insectivorous Chinese bat species ($n=281$ individuals) found paramyxovirus RNA in insectivorous *Hipposideros cineraceus, Hipposideros armiger*, and *Taphozous melanopogon* bats and in frugivorous *Eonycteris spelaea* and *Rousettus leschenaultii* (Yuan *et al.* 2014). In general, the insectivorous bats harbor paramyxoviruses that are distinct from those present in frugivorous bats. Viruses from insectivorous bats cluster within the genus *Jeilongvirus*. Henipa-related viral RNA was found in Asian *E. spelaea*, while rubulavirus RNA was detected in *R. leschenaultia* fruit bats (Yuan *et al.* 2014).

3.12.2 Multiviral paramyxoviruses studies in Africa

A large study of paramyxoviruses sequences in Africa, which included 4954 bats from 86 species, found that the majority of paramyxoviruses were present in primates, birds, carnivores, and ungulates. The number of paramyxoviruses in bats was close to that in ungulates (Drexler *et al.* 2012). This large study discovered many novel paramyxoviruses. Members of the *Morbillivirus* genus or a *Morbillivirus*-related clade were detected in 1.3% of *Hipposideros abae*, 5.0% of *Hipposideros cf caffer*, 1.7% of *Hipposideros cf ruber*,1.0% of *H. gigas*, 1.0% of *C. afra*, 8.0% of *Carollia brevicauda*, 0.3% of *Carollia perspicillata*, 13.8% of *Desmodus rotundus*, 3.4% of *Glossophaga soricina*, 5.0% of *Pteronotus parnellii*, 25.0% of *Myotis alcathoe*, 1.1% of *Myotis bechsteinii*, 11.0% of *Myotis capaccini*, 0.4% of *Myotis daubentonii*, 1.9% of *Myotis myotis*, 5.4% of *Myotis mystacinus*, and 11.1% of *Pipistrellus cf nanus/annulus*. Members of the *Rubulavirus* genus were detected in 4.0% of *Eidolon helvum*, 50.0% of *E. minimus*, 3.7% of *H. monstrosus*, 2.9% of *Megaloglossus woermanni*, 7.0% of *R. aegyptiacus*, 0.5% of *H. gigas*, and 1.6% of *M. inflatus*. Members of the *Henipavirus* genus were detected in 5.8% of *E. helvum*, 5.6% of *Epomophorus gambianus*, 5.9% of *Epomophorus* species, 3.7% of *H. monstrosus*, 2.7% of *Myonycteris torquata*, 1.4% of *R. aegyptiacus*, 0.3% of *C. perspicillata*, and 7.5% of *P. parnellii*. Members of the *Pneumovirinae* subfamily of paramyxoviruses were also detected in 1.8% of *E. helvum*. These comprise a sister clade to human and bovine respiratory syncytia viruses (Drexler *et al.* 2012). No respiroviruses were found in any of the tested bat species.

In Kenya, 5.1% of bat fecal samples contained paramyxovirus RNA ($n=217$) (Conrardy *et al.* 2014). The RNA-positive bats were members of *Cardioderma cor*, a *Chaerephon* species bat, *Otomops martiensseni, R. aegyptiacus, Miniopterus minor*, and *Miniopterus natalensis*. One bat also had paramyxovirus RNA in kidney, lung, and liver tissues.

A study of 1220 bats from 48 species in Sub-Saharan Africa discovered many diverse paramyxovirus RNA sequences of at least two major viral lineages from bat renal tissue (Mortlock *et al.* 2015). The bat species bearing viral RNA were *C. afra, Eptesicus hottentotus, Hipposideros caffer, Hipposideros fuliginosus, Kerivoula argentata, M. minor, Neoromicia nana, Nycteris thebaica, O. martiensseni, Rhinolophus*

denti, Rhinolophus landeri, Taphozous species, and *Triaenops afer* (Mortlock *et al*. 2015). The authors of this study suggested that two separate lineages might have been formed during the evolution of these bat-associated paramyxoviruses, with one lineage found in pteropodid bats and the other lineage in nonpteropodid bats. Additionally, the variation that was found in viral incidence and diversity suggest that some bat species may serve as true viral reservoirs, while other bat species are incidental hosts (Mortlock *et al*. 2015).

3.12.3 Multiviral paramyxoviruses studies in Madagascar and islands of the Southwest Indian Ocean

A study in Madagascar found that 19.2% of *E. dupreanum* ($n=73$) were seropositive for HeV and NiV as well as 2.3% of *P. rufus* ($n=349$) (Iehlé *et al*. 2007). Antibodies to Tioman virus, another paramyxovirus, were found in less than 1% of *P. rufus* and *Roussettus madagascariensis* serum samples.

A study of pooled kidney, lung, and spleen from 15 bat species from islands of the Southwest Indian Ocean isolated ten distinct paramyxoviruses from five insectivorous species (*M. gleni, M. griveaudi, Miniopterus sorculus, Mormopterus acetabulosus*, and *T. menamena*) from the Union of the Comoros (4.5% of tested bats were positive; $n=66$), Mauritius (1.8% positive; $n=55$), and Madagascar (5.4% positive; $n=76$) (Wilkinson *et al*. 2012). While all of the viral sequences were from new members of Morbillivirus-related paramyxoviruses, a high level of genetic diversity exists among the viruses, with nucleotide sequence similarity averaging 74.2%. Even in two individual *M. griveaudi* each co-infected by two distinct paramyxoviruses, the genetic identities between the pairs of viruses from the same individual bat were 70.8% and 74.7%. Eight of the novel viral sequences grouped with paramyxoviruses from insectivorous bats which inhabit large areas of Africa and Northern Madagascar, while viral sequences from *M. griveaudi* grouped with paramyxoviruses from insectivorous bat species originating in Europe (Wilkinson *et al*. 2012). Interestingly, no paramyxoviruses were detected in any tested frugivorous *Rousettus oblivious* bats ($n=36$), even though they were collected from the same location as infected insectivorous *M. griveaudi*. RNA from four paramyxoviruses was also detected in urine samples from a bat colony on the nearby Réunion Island ($n=422$) (Dietrich *et al*. 2015). Peaks of infection occurred during late pregnancy and again 2 months after the birth pulse. A unique bat-specific *Leptospira* bacterial species was also present in this colony and peaked at the same two times. Co-infection occurs frequently during times of peak transmission, however the pattern of infection does not support any interactions between the bacterial and viral pathogens. Crowded maternal bat colonies and colonies with many young juveniles that recently lost protective maternal antibodies may serve as viral transmission "hot spots" between bats and possibly to other animals having contact with the colonies at these times as well (Dietrich *et al*. 2015).

3.12.4 Multiviral paramyxoviruses studies in Oceania

A 2015 study in Australia isolated seven species of paramxyoviruses from flying-fox urine: the previously isolated HeV, Menangle virus, and Cedar virus and four novel *Rubulavirus* genus paramyxoviruses (Hervey, Grove, Teviot and Yeppoon viruses).

The number of rubulaviruses in the study was higher than any other virus genus (Barr *et al*. 2015).

In a comparison of a 1999 and a 2009 study of paramyxovirus prevalence in *P. conspicillatus* from proximate locations in Papua New Guinea, the earlier data detected no antibodies against HeV, while the latter study reported a seroprevalance rate of 65% (Field *et al*. 2013). When comparing multiple species of bats throughout Papua New Guinea, the crude seroprevalance to HeV was 7.8% in 1999, compared with 50% in 2009. Also, in 1999, almost all of the bats having neutralizing antibody against NiV had higher neutralizing titers against HeV, suggesting that the circulating henipavirus at that time was more similar to HeV than to NiV. In contrast, in the 2009 study, the virus antigens were more similar to NiV (Field *et al*. 2013). In 1999, no antibodies against Menangle virus were detected, as compared with a seroprevalance rate of 56% in 2009. Together, it appears that the prevalence of paramyxoviruses in flying foxes, including henipaviruses, is increasing in Papua New Guinea. It is also possible, however, that the sensitivity of the tests increased in the 10 years between the studies.

3.13 CONCLUSIONS

Symptomatic human infection with paramyxoviruses may result in mild, self-resolving illness to life-threatening disease, even in immunocompetent people. While pathogenic members of this group have been detected in bats and other mammals throughout the world, indirect or direct zoonotic transmission of the highly pathogenic henipaviruses in Oceania and Southeast Asia are of particular concern. About half of the tested bats from Papua New Guinea were seropositive for henipavirus species. In Africa, RNA from seven novel paramyxoviruses was found in *E. helvum*, several with a small degree of nucleic acid homology to NiV. A low percentage of humans from the area were also seropositive for henipaviruses, particularly people butchering bat bushmeat in regions with limited forest cover. Seropositive bats were also detected in Madagascar.

Henipavirus receptor binding, fusion, and entry into host cells are due to the viral G and F proteins. Glycoproteins derived from the M74 henipavirus caused syncytium formation in several cell lines from *H. monstrosus* and *E. helvum*, but not in cell lines from other mammalian species, perhaps as a result of its higher expression level on bat cell surfaces. The subsequent production of multinucleated giant cells may cause much of the henipavirus-related pathogenesis. Production of functional F protein relies upon proteolytic cleavage by highly conserved host cell enzymes, including furin. Furin from *P. alecto* differs from that of other mammals and is more active, particularly in kidney cell lines, enabling it to more rapidly process parainfluenza F protein. It should be noted that this work utilized cell lines and may therefore differ from results obtained with either primary cells or in cells infected *in vivo*.

HeV-specific antibodies have been reported in six species of flying foxes in Oceania, including all four species found throughout Australia, as well as two *Dobsonia* species. It should be noted that HeV disease has only been reported in horses or humans in tropical and subtropical regions of Australia. A nonlinear relationship exists between mean HeV excretion prevalence and five latitudinal regions. A later study suggested that *P. alecto* and *P. conspicillatus* are the most likely bat reservoir hosts since both inhabit tropical and subtropical parts of Australia. While HeV RNA was not detected in

P. poliocephalis samples, a low number of samples from this bat species did contain RNA for Yara Bend and Geelong paramyxoviruses, Teviot virus, and Cedar virus. In *P. alecto*, HeV RNA levels were highest in urine or urogenital samples, indicating that urination by this bat species may be an important route of HeV transmission to horses resting under trees containing bat roosts. Additionally, HeV RNA has been detected in several organs, including the kidneys, from *P. alecto* and *P. conspicillatus*, but not *P. scapulatus* and in only 1% of samples from *P. poliocephalus*.

The currently changing interactions between flying foxes, horses, and humans may increase the risk of zoonotic outbreaks over time as bat presence in urban areas continues to grow because most HeV outbreaks in humans occur near groups of urbanized or sedentary bat groups. Periods of time during which maternal immunity is decreasing in juvenile bats also coincide with periods of peak risk of HeV spillover into human populations

NiV infection occurs in Southeast Asia and causes severe disease in humans. Zoonotic transmission of this virus occurs by contact with pigs, perhaps by the respiratory route since NiV is present in pig upper and lower respiratory tracts. While pigs have been suggested to serve as an amplifying host, they may also be an important viral reservoir since, during one outbreak, almost all of the pigs were infected, but most were apparently healthy and the mortality rate was 5% or less. A 1998 NiV outbreak in humans in Malaysia followed an outbreak in area pigs. Anti-NiV antibodies were also detected in several fruit bat species and NiV was isolated from bats' urine and their partially eaten fruits. Pigs are believed to be infected by eating this contaminated fruit. RNA has since been detected in urine or saliva of frugivorous and insectivorous bats in Thailand; in bat urine, bladder, and oropharangyeal swabs in Sumatra; and in livers of flying foxes in India, establishing several species of bats in different areas of Southeast Asia as NiV reservoir hosts. Two strains of NiV are found in bats; the most dangerous of these not only has a very high fatality rate but may be transmitted between humans as well as directly from pigs to humans via consumption of either saliva- or urine-contaminated raw or fermented date palm sap. Henipaviruses can survive for a week or more in palm sap at 22 °C and on mango flesh, for hours to days, depending on temperature and fruit pH. The changing fruit bat diet throughout the year affects urine pH and, thus, viral survival times. Urinary pH differs even between species of flying foxes fed similar diets, perhaps affecting their capacity to transmit henipaviruses.

Nine groups of rubulaviruses have been reported in bats. Most are not known to be pathogenic to either bats or humans, despite finding antibodies in humans against several of these viruses (bat parainfluenza virus, Tioman virus, and the Achimoto viruses). Nevertheless, Jeilongvirus infection was associated with severe lung infection in one bat, Menangle virus causes a mild febrile illness with rash in humans and severe disease in pigs, and Sosuga virus infection caused high fever, headache and stiff neck, myalgia and arthralgia, maculopapular rash, and oropharynx ulcerations in a person working with bats and rodents. Several bats were found to be infected but rodent infection was not reported.

RNA from several other groups of paramyxoviruses was also detected in bats. Morbillivirus-related viruses have been in found in many very diverse bat species. The black rat is a major reservoir of this group of viruses. Belinga bat virus RNA was detected in hearts and livers of *C. afra* from Gabon. One bat was severely ill and had diarrhea, severe hemorrhagic lesions in various organs, lung congestion, and pleurisy.

Preventing human and animal infections by paramyxoviruses is a matter of great concern, especially for viruses that cause life-threatening diseases with high fatality rates, such as henipaviruses. Some of the preventative measures listed earlier have proven to be quite effective in preventing or managing paramyxovirus infections, such as placing skirts over date palm sap collection vessels, or other viral infections, such as vaccination of domestic animals (rabies), wearing proper protective equipment (Ebola), and restricting sale of known viral animal hosts (prion diseases, avian influenza, SARS). Since the vectors which introduce henipaviruses into human populations are horses and pigs, we might do well to enhance our surveillance and infection control efforts in those animals rather than wild reservoir hosts. For those microbes that, after entering a human population, may be transmitted by a human-to-human route, such as the Nipah virus, quarantine, contact tracing, and changing societal dietary and funeral practices may decrease the risk of large outbreaks in humans.

REFERENCES

Albariño CG, Foltzer M, Towner JS, Rowe LA, Campbell S, Jaramillo CM, Bird BH, Reeder DM, Vodzak ME, Rota P, Metcalfe MG, Spiropoulou CF, Knust B, Vincent JP, Frace MA, Nichol ST, Rollin PE, Ströher U. 2014. Novel paramyxovirus associated with severe acute febrile disease, South Sudan and Uganda, 2012. *Emerging Infectious Diseases.* 20(2):211–214.

Amman BR, Albariño CJ, Bird BH, Nyakarahuka L, Sealy TK, Balinandi S, Schuh AJ, Campbell SM, Ströher U, Jones MEB, Vodzack ME, Reeder DM, Kaboyo W, Nichol ST, Towner JS. 2015. A recently discovered pathogenic paramyxovirus, Sosuga virus, is present in *Rousettus aegyptiacus* fruit bats at multiple locations in Uganda. *Journal of Wildlife Diseases.* 51(3):774–779.

Baker KS, Todd S, Marsh Ga, Crameri G, Barr J, Kamins AO, Peel AJ, Yu M, Hayman DTS, Nadjm B, Mtove G, Amos B, Reyburn H, Nyarko E, Suu-Ire R, Murcia PR, Cunningham AA, Wood JLN, Wang L-F. 2013. Novel, potentially zoonotic paramyxoviruses from the African straw-colored fruit bat *Eidolon helvum*. *Journal of Virology.* 87(3):1348–1358.

Barr J, Smith C, Smith I, de Jong C, Todd S, Melville D, Broos A, Crameri S, Haining J, Marsh G, Crameri G, Field H, Wang LF. 2015. Isolation of multiple novel paramyxoviruses from pteropid bat urine. *Journal of General Virology.* 96(Pt 1):24–29.

Basler CF. 2012. Nipah and Hendra virus interactions with the innate immune system. *Current Topics in Microbiology and Immunology.* 359:123–152.

Breed AC, Breed MF, Meers J, Field HE. 2011. Evidence of endemic Hendra virus infection in flying-foxes (*Pteropus conspicillatus*) – implications for disease risk management. *PLoS ONE.* 6(12):e28816.

Breed AC, Meers J, Sendow I, Bossart KN, Barr JA, Smith I, Wacharapluesadee S, Wang L, Field HE. 2013. The distribution of henipaviruses in Southeast Asia and Australasia: is Wallace's line a barrier to Nipah virus? *PLoS ONE.* 8(4):e61316.

Breed AC, Yu M, Barr JA, Crameri G, Thalmann CM, Wang L-F. 2010. Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. *Emerging Infectious Diseases.* 16(12):1997–1999.

Burroughs AL, Durr PA, Boyd V, Graham K, White JR, Todd S, Barr J, Smith I, Baverstock G, Meers J, Crameri G, Wang LF. 2016. Hendra virus infection dynamics in the grey-headed flying fox (*Pteropus poliocephalus*) at the southern-most extent of its range: further evidence this species does not readily transmit the virus to horses. *PLoS ONE.* 11(6):e0155252.

CDC. 2016. Signs & symptoms of mumps. https://www.cdc.gov/mumps/index.html. Accessed May 15, 2017.

Chakraborty A, Sazzad HM, Hossain MJ, Islam MS, Parveen S, Husain M, Banu SS, Podder G, Afroj S, Rollin PE, Daszak P, Luby SP, Rahman M, Gurley ES. 2016. Evolving epidemiology of Nipah virus infection in Bangladesh: evidence from outbreaks during 2010-2011. *Epidemiology & Infection.* 144(2):371–380.

Ching PKG, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF Jr, Bolo GC Jr, Sejvar JJ, Eagles D, Playford G, Dueger E, Kaku Y, Morikawa S, Kuroda M, Marsh GA, McCullough S, Foxwell AR. 2015. Outbreak of Henipavirus infection, Philippines, 2014. *Emerging Infectious Diseases.* 21:328–331.

Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes and Infections.* 4(2):145–151.

Chua KB, Wang LF, Lam Sai Kit K, Crameri GS, Yu M, Wise T, Boyle DB, Hyatt AD, Eaton BT. 2001. Tioman virus, a novel paramyxovirus isolated from fruit bats in Malaysia. *Virology.* 283:215–229.

Clayton BA, Wang LF, Marsh GA. 2013. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and Public Health.* 60:69–83.

Conrardy C, Tao Y, Kuzmin IV, Niezgoda M, Agwanda B, Breiman RF, Anderson LJ, Rupprecht C, Tong S. 2014. Molecular detection of adenoviruses, rhabdoviruses, and paramyxoviruses in bats from Kenya. *American Journal of Tropical Medicine and Hygiene.* 91(2):258–266.

de Wit E, Prescott J, Falzarano D, Bushmaker T, Scott D, Feldmann H, Munster VJ. 2014. Foodborne transmission of Nipah virus in Syrian hamsters. *PLoS Pathology.* 10:e1004001.

Dietrich M, Wilkinson DA, Benlali A, Lagadec E, Ramasindrazana B, Dellagi K, Tortosa P. 2015. *Leptospira* and paramyxovirus infection dynamics in a bat maternity enlightens pathogen maintenance in wildlife. *Environmental Microbiology*. 17(11):4280–4289.

Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, Ipsen A, Kruppa T, Müller MA, Kalko EK, Adu-Sarkodie Y, Oppong S, Drosten C. 2009. Henipavirus RNA in African bats. *PLoS ONE.* 4(7):e6367.

Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Cottontail VM, Rasche A, Yordanov S, Seebens A, Knörnschild M, Oppong S, Adu Sarkodie Y, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJ, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK,Kruppa T, Franke CR, Kallies R, Yandoko ER, Herrler G, Reusken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG, Leroy EM, Drosten C. 2012. Bats host major mammalian paramyxoviruses. *Nature Communications.* 3:796.

Edson D, Field H, McMichael L, Jordan D, Kung N, Mayer D, Smith C. 2015a. Flying-Fox roost disturbance and Hendra virus spillover risk. *PLoS ONE.* 10(5):e0125881.

Edson D, Field H, McMichael L, Vidgen M, Goldspink L, Broos A, Melville D, Kristoffersen J, de Jong C, McLaughlin A, Davis R, Kung N, Jordan D, Kirkland P, Smith C. 2015b. Routes of Hendra virus excretion in naturally-infected flying-foxes: implications for viral transmission and spillover risk. *PLoS ONE.* 10(10):e0140670.

El Najjar F, Lampe L, Baker ML, Wang LF, Dutch RE. 2015. Analysis of cathepsin and furin proteolytic enzymes involved in viral fusion protein activation in cells of the bat reservoir host. *PLoS ONE.* 10(2):e0115736.

Epstein JH, Baker ML, Zambrana-Torrelio C, Middleton D, Barr JA, Dubovi E, Boyd V, Pope B, Todd S, Crameri G, Walsh A, Pelican K, Fielder MD, Davies AJ, Wang LF, Daszak P. 2013. Duration of maternal antibodies against canine distemper virus and Hendra virus in pteropid bats. *PLoS ONE.* 8(6):e67584.

Epstein JH, Prakash V, Smith CS, Daszak P, McLaughlin AB, Meehan G, Field HE, Cunningham AA. 2008. Henipavirus infection in fruit bats (*Pteropus giganteus*), India. *Emerging Infectious Diseases.* 14(8):1309–1311.

Field H, de Jong CE, Halpin K, Smith CS. 2013. Henipaviruses and fruit bats, Papua New Guinea. *Emerging Infectious Diseases.* 19(4):670–671.

Field H, Jordan D, Edson D, Morris S, Melville D, Parry-Jones K, Broos A, Divljan A, McMichael L, Davis R, Kung N, Kirkland P, Smith C. 2015. Spatiotemporal aspects of Hendra virus infection in Pteropid bats (flying-foxes) in eastern Australia. *PLoS ONE.* 10(12):e0144055.

Fogarty R, Halpin K, Hyatt AD, Daszak P, Mungall BA. 2008. Henipavirus susceptibility to environmental variables. *Virus Research.* 132(1–2):140–144.

Goldspink LK, Edson DW, Vidgen ME, Bingham J, Field HE, Smith CS. 2015. Natural Hendra virus infection in flying-foxes – tissue tropism and risk factors. *PLoS ONE.* 10(6):e0128835.

Gupta M, Lo MK, Spiropoulou CF. 2013. Activation and cell death in human dendritic cells infected with Nipah virus. *Virology.* 441:49–56.

Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK, Islam MR, Molla MA, Carroll DS, Ksiazek TG, Rota PA, Lowe L, Comer JA, Rollin P, Czub M, Grolla A, Feldmann H, Luby SP, Woodward JL, Breiman RF. 2007. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerging Infectious Diseases.* 13(7):1031–1037.

Hayman DT, Wang LF, Barr J, Baker KS, Suu-Ire R, Broder CC, Cunningham AA, Wood JL. 2011. Antibodies to henipavirus or henipa-like viruses in domestic pigs in Ghana, West Africa. *PLoS ONE.* 6(9):e25256.

Hayman DTS, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JLN, Cunningham AA. 2008. Evidence of henipavirus infection in West African fruit bats. *PLoS ONE.* 3(7):e2739.

Health Communities.com. 2016. Canine distemper. http://www.healthcommunities.com/canine-distemper/transmission.shtml. Accessed October 20, 2016.

Hollinger FB, Pavri KM. 1971. Bat parainfluenza virus: immunological, chemical, and physical properties. *American Journal of Tropical Medicine and Hygiene.* 20:131–138.

Hsu VP, Hossain MJ, Parashar UD, Ali MM, Ksiazek TG, Kuzmin I, Niezgoda M, Rupprecht C, Bresee J, Breiman RF. 2004. Nipah virus encephalitis reemergence, Bangladesh. *Emerging Infectious Diseases.* 10(12):2082–2087.

Iehlé C, Razafitrimo G, Razainirina J, Andriaholinirina N, Goodman SM, Faure C, Georges-Courbot MC, Rousset D, Reynes JM. 2007. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. *Emerging Infectious Diseases.* 13(1):159–161.

Islam MS, Sazzad HM, Satter SM, Sultana S, Hossain MJ, Hasan M, Rahman M, Campbell S, Cannon DL, Ströher U, Daszak P, Luby SP, Gurley ES. 2016. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011–2014. *Emerging Infectious Diseases.* 22(4):664–670.

Johara MY, Field H, Rashdi AM, Morrisy C. van der Heide B, Rota P, Adzhar A,White J, Daniels P, Jamaluddin A, Ksiazek TG. 2001. Nipah virus infection in bats (Order Chiroptera) in Peninsular Malaysia. *Emerging Infectious Diseases.* 7:439–441.

Khan SU, Gurley ES, Hossain MJ, Nahar N, Sharker MA, Luby SP. 2012. A randomized controlled trial of interventions to impede date palm sap contamination by bats to prevent Nipah virus transmission in Bangladesh. *PLoS ONE.* 7(8):e42689.

Krüger N, Hoffmann M, Drexler JF, Müller MA, Corman VM, Drosten C, Herrler G. 2014. Attachment protein G of an African bat henipavirus is differentially restricted in chiropteran and nonchiropteran cells. *Journal of Virology.* 88(20):11973–11980.

Krüger N, Hoffmann M, Drexler JF, Müller MA, Corman VM, Sauder C, Rubin S, He B, Örvell C, Drosten C, Herrler G. 2015. Functional properties and genetic relatedness of the fusion and

hemagglutinin-neuraminidase proteins of a mumps virus-like bat virus. *Journal of Virology.* 89(8):4539–4548.

Kurth A, Kohl C, Brinkmann A, Ebinger A, Harper JA, Wang L-F, Wühldorfer K, Wibbelt G. 2012. Novel paramyxoviruses in free-ranging European bats. *PLoS ONE.* 7(6):e38688.

Lau SK, Woo PC, Wong BH, Wong AY, Tsoi HW, Wang M, Lee P, Xu H, Poon RW, Guo R, Li KS, Chan KH, Zheng BJ, Yuen KY. 2010. Identification and complete genome analysis of three novel paramyxoviruses, Tuhoko virus 1, 2 and 3, in fruit bats from China. *Virology.* 404(1):106–116.

Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. 2015. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proceedings of the National Academy of Science U S A.* 112(17):E2156–2165.

Lieu KG, Marsh GA, Wang L-F, Netter HJ. 2015. The nonpathogenic henipavirus Cedar paramyxovirus phosphate has a compromised ability to target STAT12 and STAT2. *Antiviral Research.* 124:69–76.

Lo Presti A, Cella E, Giovanetti M, Lai A, Angeletti S, Zehender G, Ciccozzi M. 2016. Origin and evolution of Nipah virus. *Journal of Medical Virology.* 88(3):380–388.

Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA, Ksiazek TG. 2006. Foodborne transmission of Nipah virus, Bangladesh. *Emerging Infectious Diseases.* 12(12):1888–1894.

Mackenzie JS. 1999. Emerging viral diseases: An Australian perspective. *Emerging Infectious Diseases.* 5(1):1–8.

Maganga GD, Bourgarel M, Obame Nkoghe J, N'Dilimabaka N, Drosten C, Paupy C, Morand S, Drexler JF, Leroy EM. 2014. Identification of an unclassified paramyxovirus in *Coleura afra*: a potential case of host specificity. *PLoS ONE* 9(12):e115588.

Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, Middleton D, Yu M, Todd S, Foord AJ, Haring V, Payne J, Robinson R, Broz I, Crameri G, Field HE, Wang L-F. 2012. Cedar virus: a novel henipavirus isolated from Australian bats. *PLoS Pathology.* 8(8): e1002836.

Mayo Clinic. 2016. Measles. http://www.mayoclinic.org/diseases-conditions/measles/basics/symptoms/con-20019675. Accessed October 20, 2016.

MedLine Plus. 2016. Parainfluenza viruses. https://medlineplus.gov/ency/article/001370.htm. Accessed October 20, 2016.

Mélade J, Wieseke N, Ramasindrazana B, Flores O, Lagadec E, Gomard Y, Goodman SM, Dellagi K, Pascalis H. 2016. An eco-epidemiological study of Morbilli-related paramyxovirus infection in Madagascar bats reveals host-switching as the dominant macro-evolutionary mechanism. *Scientific Reports.* 6:23752.

Merck Veterinary Manual. 2016. Rinderpest. http://www.merckvetmanual.com/. Accessed October 20, 2016.

Middleton DJ, Weingartl HM. 2012. Henipaviruses in their natural animal hosts. *Current Topics in Microbiology and Immunology.* 359:105–121.

Mortlock M, Kuzmin IV, Weyer J, Gilbert AT, Agwanda B, Rupprecht CE, Nel LH, Kearney T, Malekani JM, Markotter W. 2015. Novel paramyxoviruses in bats from Sub-Saharan Africa, 2007–2012. *Emerging Infectious Diseases.* 21(10):1840–1843.

Muleya W, Sasaki M, Orba Y, Ishii A, Thomas Y, Nakagawa E, Ogawa H, Hang'ombe B, Namangala B, Mweene A, Takada A, Kimura T, Sawa H. 2014. Molecular epidemiology of paramyxoviruses in frugivorous *Eidolon helvum* bats in Zambia. *Journal of Veterinary Medical Science.* 76(4):611–614.

Nahar N, Paul RC, Sultana R, Gurley ES, Garcia F, Abedin J, Sumon SA, Banik KC, Asaduzzaman M, Rimi NA, Rahman M, Luby SP. 2015. Raw sap consumption habits and its association with knowledge of Nipah virus in two endemic districts in Bangladesh. *PLoS ONE.* 10(11):e0142292.

Ong KC, Wong KT. 2015. Henipavirus encephalitis: recent developments and advances. *Brain Pathology.* 25(5):605–613.

Pavri KM, Singh KR, Hollinger FB. 1971. Isolation of a new parainfluenza virus from a frugivorous bat, *Rousettus leschenaulti*, collected at Poona, India. *American Journal of Tropical Medicine and Hygiene*. 20:125–130.

Peel AJ, Baker KS, Crameri G, Barr JA, Hayman DT, Wright E, Broder CC, Fernández-Loras A, Fooks AR, Wang LF, Cunningham AA, Wood JL. 2012. Henipavirus neutralising antibodies in an isolated island population of African fruit bats. *PLoS ONE.* 7(1):e30346.

Pernet O, Schneider BS, Beaty SM, LeBreton M, Yun TE, Park A, Zachariah TT, Bowden TA, Hitchens P, Ramirez CM, Daszak P, Mazet J, Freiberg AN, Wolfe ND, Lee B. 2014. Evidence for henipavirus spillover into human populations in Africa. *Nature Communucations.* 5:5342.

Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, Daniels PW, Gould AR, Hyatt AD. 1998. An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats. *Emerging Infectious Diseases.* 4(2):269–271.

Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, Daszak P, Foley JE. 2008. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of Biological Sciences*. 275(1636):861–869.

Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, Daszak P. 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proceedings in Biological Sciences*. 278(1725):3703–3712.

Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, Rahman M, Gurley ES, Rollin PE, Lo MK, Comer JA, Lowe L, Rota PA, Ksiazek TG, Kenah E,Sharker Y, Luby SP. 2012. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Diseases.* 12(1):65–72.

Rodriguez JJ, Parisien JP, Horvath CM. 2002. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *Journal of Virology.* 76(22):11476–11483.

Rodriguez JJ, Wang LF, Horvath CM. 2003. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *Journal of Virology.* 77(21):11842–11845.

Sasaki M, Setiyono A, Handharyani E, Rahmadani I, Taha S, Adiani S, Subangkit M, Sawa H, Nakamura I, Kimura T. 2012. Molecular detection of a novel paramyxovirus in fruit bats from Indonesia. *Virology Journal.* 9:240.

Sendow I, Field HE, Curran J, Darminto, Morrissy C, Meehan G, Buick T, Daniels P. 2006. Henipavirus in *Pteropus vampyrus* bats, Indonesia. *Emerging Infectious Diseases.* 12(4):711–712.

Sendow I, Ratnawati A, Taylor T, Adjid RM, Saepulloh M, Barr J, Wong F, Daniels P, Field H. 2013. Nipah virus in the fruit bat *Pteropus vampyrus* in Sumatera, Indonesia. *PLoS ONE.* 8(7):e69544.

Shaw ML. 2009. Henipaviruses employ a multifaceted approach to evade the antiviral interferon response. *Viruses.* 1(3):1190–1203.

Smith L, Wang L-F. 2013. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Current Opinion in Virology.* 3:84–91.

Virtue ER, Marsh GA, Baker ML, Wang L-F. 2011a. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS ONE.* 6(7):e22488.

Virtue ER, Marsh GA, Wang LF. 2011b. Interferon signaling remains functional during henipavirus infection of human cell lines. *Journal of Virology.* 85(8):4031–4034.

Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P, Saengsen D, Gongal GN, Hemachudha T.2010. A longitudinal study of the prevalence of Nipah virus in

Pteropus lylei bats in Thailand: evidence for seasonal preference in disease transmission. *Vector Borne Zoonotic Diseases.* 10:183–190.

Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, Stockton P, Rupprecht CE, Ksiazek TG, Hemachudha T. 2005. Bat Nipah virus, Thailand. *Emerging Infectious Diseases.* 11(12):1949–1951.

Wacharapluesadee S, Samseeneam P, Phermpool M, Kaewpom T, Rodpan A, Maneeorn P, Srongmongkol P, Kanchanasaka B, Hemachudha T. 2016. Molecular characterization of Nipah virus from *Pteropus hypomelanus* in Southern Thailand. *Virology Journal.* 13(1):53.

Weis M, Behner L, Hoffmann M, Krüger N, Herrler G, Drosten C, Drexler JF, Dietzel E, Maisner A. 2014. Characterization of African bat henipavirus GH-M74a glycoproteins. *Journal of General Virology.* 95(Pt 3):539–548.

Wilkinson DA, Temmam S, Lebarbenchon C, Lagadec E, Chotte J, Guillebaud J, Ramasindrazana B, Héraud JM, de Lamballerie X, Goodman SM, Dellagi K, Pascalis H. 2012. Identification of novel paramyxoviruses in insectivorous bats of the Southwest Indian Ocean. *Virus Research.* 170(1–2):159–163.

Wynne JW, Shiell BJ, Marsh GA, Boyd V, Harper JA, Heesom K, Monaghan P, Zhou P, Payne J, Klein R, Todd S, Mok L, Green D, Bingham R, Tachedjian M, Baker ML, Matthews D, Wang L-F. 2014. Proteomics informed by transcriptomics reveals Hendra virus sensitizes bat cells to TRAIL-mediated apoptosis. *Genome Biology*.15:532.

Yadav PD, Raut CG, Shete AM, Mishra AC, Towner JS, Nichol TS, Mourya DT. 2012. Short report: detection of Nipah virus RNA in fruit bat (*Pteropus giganteus*) from India. *American Journal of Tropical Medicine and Hygiene.* 87(3): 576–578.

Yaiw KC, Crameri G, Wang L, Chong HT, Chua KB, Tan CT, Goh KJ, Shamala D, Wong KT. 2007. Serological evidence of possible human infection with Tioman virus, a newly described paramyxovirus of bat origin. *Journal of Infectious Diseases.* 196(6):884–886.

Yuan L, Li M, Li L, Monagin C, Chmura AA, Schneider BS, Epstein JH, Mei X, Shi Z, Daszak P, Chen J. 2014. Evidence for retrovirus and paramyxovirus infection of multiple bat species in China. *Viruses*. 6(5):2138–2154.

Zeller HG, Karabatsos N, Calisher CH, Digoutte JP, Murphy FA, Shope RE. 1989. Electron microscopy and antigenic studies of uncharacterized viruses. I. Evidence suggesting the placement of viruses in families Arenaviridae, Paramyxoviridae, or Poxviridae. *Archives of Virology*. 108(3–4):191–209.

Zhou P, Cowled C, Todd S, Crameri G, Virtue ER, Marsh GA, Klein R, Shi Z, Wang L-F, Baker ML. 2011. Type III IFNs in pteropid bats: differential expression patterns provide evidence for distinct roles in antiviral immunity. *Journal of Immunology.* 186(5):3138–3147.

4

FILOVIRUSES AND BATS

4.1 FILOVIRUSES

The Filoviridae family consists of pleomorphic, filamentous, single-stranded, negative-sense, non-segmented RNA viruses. The filaments have a uniform diameter of 80 nm and may be long (up to 12 000 nm) and branched or shorter and shaped like a "6," a "U," or a circle. The family belongs to the order *Mononegavirales* and contains three genera: *Marburgvirus* (MARV); *Ebolavirus*; and *Cuevavirus*. *Ebolavirus* contains five species: *Zaire ebolavirus* (EBOV); *Sudan ebolavirus* (SEBOV); *Côte d'Ivoire ebolavirus* (CIEBOV); *Reston ebolavirus* (REBOV); and *Bundibungyo ebolavirus* (BEBOV). While REBOV infects humans, it is not pathogenic, although it causes a lethal disease in some nonhuman primates. MARV (Lake Victoria *marburgvirus*) is the sole species of the genera and contains Marburg and Ravn viruses, with approximately 20% genetic divergence. *Lloviu cuevavirus* (Lloviu virus) is the sole member of its genus and infectious virus has yet to be isolated, forcing researchers to utilize pseudotyped viruses bearing Lloviu proteins when studying infection of host cells (Maruyuma *et al*. 2014). It is genetically equally distinct from ebolaviruses and MARV (approximately 50% sequence divergence from these genera). It utilizes pathways of host cell entry similar to other filoviruses, but is more comparable with ebolaviruses than to MARV, as described below (Maruyuma *et al*. 2014; M. Ng *et al*. 2014). These similarities and differences with other filoviruses support the contention that LLOV belongs to a unique genus of filoviruses.

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 4.1 Filoviruses associated with bats

Bat family	Bat common name	Bat species	Filovirus
Pteropodidae	Golden-capped fruit bat	*Acerodon jubatus*	REBOV
Pteropodidae	Short-nosed fruit bat	*Cynopterus* species	EBOV
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	EBOV
Pteropodidae	Büttikofer's epauletted fruit bat	*Epomops buettikoferi*	EBOV
Pteropodidae	Büttikofer's epauletted fruit bat	*Epomops buettikoferi*	MARV
Pteropodidae	Franquet's epauletted fruit bat	*Epmops franqueti*	ZEBOV
Pteropodidae	Franquet's epauletted fruit bat	*Epomops franqueti*	MARV
Pteropodidae	Hammerhead bat	*Hypsignathus monstrosus*	ZEBOV
Pteropodidae	Hammerhead bat	*Hypsignathus monstrosus*	MARV
Megadermatidae	Greater false vampire bat	*Megaderma lyra*	EBOV
Pteropodidae	Peter's dwarf epauletted fruit bat	*Micropteropus pusillus*	EBOV
Pteropodidae	Peter's dwarf epauletted fruit bat	*Micropteropus pusillus*	MARV
Miniopteridae	Greater long-fingered bat	*Miniopterus inflatus*	MARV
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Lloviu virus
Molossidae	Angolan free-tailed bats	*Mops condylurus*	EBOV
Pteropodidae	Little collared fruit bat	*Myonycteris torquata*	ZEBOV
Pteropodidae	Large flying fox	*Pteropus vampyrus*	REBOV
Rhinolophidae	Eloquent horseshoe bat	*Rhinolophus eloquens*	MARV
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	EBOV
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	MARV
Pteropodidae	Geoffroy's rousette	*Rousettus amplexicaudatus*	REBOV
Pteropodidae	Leschenault's rousette	*Rousettus leschenaulti*	Bt-DH04 filovirus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaulti*	EBOV

Prior to the 2014–2015 outbreak in Western Africa, ecologic niche modeling of outbreaks suggested that Ebola hemorrhagic fever was present in rain forests of central and western Africa, while Marburg was found in drier, more open areas of south central and eastern Africa (reviewed in Beltz 2011). Working in or entering mines, decommissioned mines, and caves are risk factors for human infection with MARV. While the majority of the above filoviruses reside in Africa, REBOV originates in the Philippines and Lloviu virus is found in Spain. Filoviruses that have been reported to be associated with bats are found in Table 4.1.

4.1.1 History of filovirus infection

For information prior to the West African EBOV outbreak of 2014–2015, Mylne *et al.* (2014) compiled a comprehensive database of the geographic spread of human Ebola outbreaks that extracted details of suspected zoonotic origins and human-to-human spread utilizing a variety of published and non-published sources. This information may be of great use in future outbreaks in Central Africa and for comparative purposes between the outbreaks in different regions of the continent.

4.1.2 Filovirus disease

Following an incubation period of 2–42 days, disease onset is abrupt, beginning with nonspecific, influenza-like symptoms, including high fever, headache, joint and muscle ache, nausea, and sore throat, followed by intense fatigue, diarrhea, abdominal pain, and vomiting. Skin manifestations include petechiae and purpural skin rash (red or purple spots on the skin). Early during the course of infection, monocyte/macrophages and dendritic cells of the immune system become infected. The viruses subsequently travel via the blood and lymphatic systems to the liver, spleen, and other organs (Olejnik *et al*. 2011). Respiratory symptoms include cough, hiccups, throat and chest pain, and difficulty breathing. Severe disease manifestations include necrosis of the liver, spleen, kidney, lymph nodes, testes, and ovaries, resulting from viral replication within parenchymal cells. Injury to the microvascular tissue induces increased vascular permeability and activation and depletion of elements of the clotting cascade with eventual disruption of fluid balance, cardiovascular distress, hypovolemic shock, and death (Rewar & Mirdha 2014). The fatality rate in humans may be quite high for some of these viruses, 30–90%, while the REBOV is asymptomatic in humans.

4.1.3 The roles of viral proteins

Understanding how viruses evade the immune response and enter host cells is critical to comprehending viral persistence as well as cellular and species tropism. It is important to determine both the viral and host cell proteins involved, since changes in either of these may increase or decrease infection of various host cell types and subsequent development and severity of disease. The filoviral genome contains genes for the following proteins, in 3′ to 5′ order: nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), replication-transcription protein (VP30), minor matrix protein (VP24 – unique to filoviruses), RNA-dependent RNA polymerase (L), and the secreted glycoprotein (sGP), which serves an anti-inflammatory function by protecting the endothelium of vessel walls (Takada 2012).

4.1.3.1 The role of viral and cellular proteins in evading the host immune response VP35 and VP24 are virulence factors that decrease the host IFN response (Olejnik *et al*. 2011). Severe disease due to the ebolavirus or Marburg filovirus infection in humans is associated with dysregulated innate and adaptive immunity by decreasing action of type I and type II IFN, critical elements of bats' antiviral defenses, as discussed in Chapter 1. The highly pathogenic ZEBOV and MARV are far better at inhibiting IFN-α and -β function than is the nonpathogenic REBOV. The VP35 of ZEBOV and MARV is critical to blocking IFN-α and IFN-β production by the RIG-I cellular RNA helicase pathway and subsequent downstream signaling, while VP24 inhibits IFN-α/β and IFN-γ signaling.

VP35 serves as a pseudosubstrate for several cellular kinases, IKKε (IκB kinase ε) and TBK-1 (TANK-binding kinase 1), and blocks activation of interferon regulatory factor 3 (IRF-3) and IRF-7. VP35 also promotes chemical modification of IRF-7, which represses IFN gene transcription by a negative feedback loop. Additionally, VP35 decreases activation of PKR, a dsRNA-activated protein kinase, which inhibits virus translation and replication by phosphorylating the α-subunit of the translation initiation

factor eIF-2. Ebolavirus VP24 acts, at least in part, by preventing nuclear import of activated, phosphorylated STAT1 homodimers (in the case of type I IFN) or STAT1:STAT2 heterodimers (IFN-γ) during ebolavirus infection. The virus-induced chemokines interleukin (IL)-8 and IL-10 attract immune cells to the site of infection, where they also become infected and secrete additional chemokines and cytokines, including the proinflammatory cytokines IL-6 and IFN-γ. This also alters levels of IP-10 and the anti-inflammatory cytokine TGF-β (reviewed by Basler & Amarasinghe 2009). By contrast, MARV VP24 protein inhibits STAT1 and STAT2 phosphorylation and activation rather than STAT1 import. ZEBOV blocks IFN-α and IFN-γ from inducting expression of IRF-1 and 2′,5′-oligoadenylate synthetase, but not induction of NF-κB transcription factor complexes or IL-1β-induced gene expression (reviewed by Basler & Amarasinghe 2009). Together, these actions decrease type I and type II interferons and encourage a proinflammatory state. Some of the major components of the bat anti-viral defense system are the various type III IFNs, which play a minor role in human immunity. Neither the effects of filoviruses upon type III IFNs nor their effects upon inflammations have been reported.

4.1.3.2 The role of viral and cellular proteins in tropism and host cell entry In order to enter its host cell, either GP, the sole viral surface protein, or viral membrane phosphatidylserine bind to receptors on the host cell, either a C-type lectin or the T-cell immunoglobulin and mucin domain 1 (TIM-1), respectively. Viral entry occurs via several types of endocytosis, including macropinocytosis and clathrin- or caveola/lipid raft-mediated mechanisms. The viral envelope then fuses intracellularly with the cellular endosomal membrane. The cellular molecules of the homotypic fusion and vacuole protein-sorting multi-subunit tethering complex play a vital role in fusion of endosomes and lysosomes. The endo/lysosomal cholesterol transporter protein Niemann-Pick C1 (NPC1) is important in viral fusion and uncoating. Viral mutations can inhibit cellular infection by filoviruses as can mutations in host genes involved in the production of endosomes or lysosomes, as described below (Carette *et al*. 2012; Ng *et al*. 2015).

Following fusion with endosomes, the viral proteins and genome are released into the cell's cytoplasm, where filoviruses then replicate and produce mRNAs for viral protein synthesis. Genomic RNA, surrounded by its NP capsid, combines with viral proteins during viral assembly at the host cell's plasma membrane. Viral particles then bud from the surface, forming the enveloped viral progeny (Takada 2012).

Filoviruses infect and grow in a large variety of primate cell types, including Vero cells (a kidney cell line from African green monkeys), but have a special affinity for liver hepatocytes and Kupffer cells, adrenal cortical cells, fibroblasts, endothelial cells, and, importantly, dendritic cells and monocyte/macrophages of the innate immune system. Infection of the latter cells is important for virulence, including hemorrhagic manifestations, dysfunction of early innate immune action, and viral dissemination throughout the body. While lymphocytes are not infected, filovirus infection depletes uninfected lymphocytes by apoptosis, adversely affecting the adaptive immune response, interfering with vial clearance (Olejnik *et al*. 2011; Takada 2012). Data from survivors indicates that an early and properly regulated cytokine response may be critical in determining disease outcome (Olejnik *et al*. 2011). In bats' livers, MARV antigen is found in a perimembranous pattern around small, relatively isolated foci, often associated with small numbers of mononuclear inflammatory cells and highly localized hepatocyte

necrosis (Towner 2009). This stands in sharp contrast to the abundant and extensively distributed antigens seen in infected humans and non-human primates livers (reviewed in Towner *et al*. 2009).

When the R06E cell line from *Rousettus aegyptiacus* bats was exposed to EBOV or MARV, the cells were infected and had similar growth kinetics (MARV) and produced infectious filoviral titers similar to those produced in Vero cells (both MARV and EBOV) (Krähling *et al*. 2010). While mature viral particles budded from the plasma membrane and filamentous particles were found in the supernatant of the R06E cell cultures, R06E cells' cytoplasm also demonstrated large numbers of intracellular nucleocapsids characteristic of filoviruses, more than seen in Vero cells or human cell lines. The viral inclusions in R06E cells, indicative of accumulations of viral nucleocapsids, were also larger than those present in Vero cells (Krähling *et al*. 2010). Bat-derived R06E cells, thus, may release viral particles less efficiently than the other cell lines, suggesting a need to further study viral budding efficiency and interactions between viral proteins and components of the endosomal sorting complex required for transport in primary cell cultures from a variety of bat groups infected *in vivo*. Such studies may clarify the importance of the role of the interaction of a MARV protein or the corresponding EBOV protein (VP40) with cellular TSG101 alone or TSG101 together with Nedd4 during filovirus budding (Licata *et al*. 2003; Timmins *et al*. 2003; Urata *et al*. 2007). The fact that the R06E bat cell line expresses high levels of viral proteins and high viral titers are found in the supernatants does not disqualify *R. aegyptiacus* as a filovirus reservoir since experimental infection of these bats with EBOV leads to productive infection, with high viral titers of virus but no clinical symptoms (Swanepoel *et al*. 1996), perhaps due to a rapid immune response in some of the bats' tissues.

Interestingly, pseudotyped viruses expressing several strains of MARV GP did not infect kidney cells derived from *Pteropus dasymallus yayeyamae* bats, although they, also as well as ebolaviruses and LLOV, did infect kidney cell lines derived from several other bat species (*Rhinolophus ferrumequinum, Miniopterus fuliginosus, Rousettus leschenaulti, Epomophorus gambianus*, and *Miniopterus schreibersi*) as well as *Pteropus giganteus* spleen cells. This suggests that cellular receptors/co-receptors may exist that interact with EBOV, REBOV, and LLOV GP, but not MARV GP (Maruyama *et al*. 2014).

The filoviral GP is active in receptor binding and fusion of the viral envelope with the host cell. Its mucin-like region (MLR), containing a large number of N- and O-linked glycans, seems to be particularly important in viral binding and entry into human cells, but is not absolutely required for viral entry into cells *in vitro*. The amino acid sequences in the MLR and the associated sugar chains vary greatly among filovirus species and among viruses propagated in different cell lines (Takada 2012). The MLR also appears to be the target of antibody-dependent enhancement, a process by which viral entry into host cells is enhanced by host antibody, in contrast to the role of neutralizing antibodies, which block cellular infection. It is not known whether antibody-dependent enhancement occurs in bats as well.

GP is cleaved by host proteases, including furin, into two subunits, GP1 and GP2, linked by a disulfide bond. GP1 mediates viral attachment, most likely through the MLR or a receptor-binding region, while GP2 contains heptad repeat regions required to assemble GP as a trimer. GP2's hydrophobic fusion loop may catalyze fusion of the viral envelope and host cell membrane, following a conformational change. Proteolysis

of viral GP by host endocytic cysteine proteases, such as cathepsins B and L or thermolysin, induces this necessary conformational change (Takada 2012). It is not known whether the bat homologs of these proteases cleave filovirus GP.

The host cell receptor(s) are not completely defined since GP interacts with multiple molecules during binding and cell entry (Takada 2012). Viral cellular tropism does not always match the distribution of any of the putative cellular molecules yet identified. It is also unclear whether these molecules mediate both viral attachment and membrane fusion or only membrane fusion. Several groups of molecules are important for cellular infection. The first molecule believed to be active in filovirus infection of human cells is TIM-1. Plasma membrane expression of TIM-1 correlates with permissiveness for all ebolaviruses and MARV by binding to phosphatidylserine of the viral envelope in a GP-independent fashion. Normally, TIM-1 recognizes host phosphatidylserine exposed on apoptotic cells and aids in their clearance via phagocytosis. The binding of TIM-1 to viral phosphatidylserine and the subsequence attachment and uptake of filoviruses is known as apoptotic mimicry (Kurdora *et al*. 2015). Filovirus in TIM-1-containing vesicles are then brought into the cell and transition into early, and then late, endosomes and endolysosomes (Kuroda *et al*. 2015). Ectopic expression of TIM-1 in poorly permissive cells enhances ebolavirus infection and reducing its cell-surface expression decreases infection of highly permissive cells (Takada 2012; Kuroda *et al*. 2015).

In cells that lack TIM-1, such as macrophages, members of the human Tyro3 receptor tyrosine kinase family (Axl, Dtk, and Mer) compose a group that allows viral-host cell attachments (Kuroda *et al*. 2015). Tyro3-family members are widely distributed in many types of cells throughout the body, but not on lymphocytes or granulocytes (Takada 2012). Decreasing cellular expression of Axl inhibits viral entry via macropinocytosis, but not by other endocytic pathways. Artificially expressing these molecules on lymphoid cells enhances infection by filovirus GP-expressing pseudoviruses.

Membrane-anchored cellular C-type lectins provide a second pathway for filovirus attachment to the human host cell plasma membrane *in vitro* in a GP-dependent pathway. C-type lectins compose a family of Ca^{2+}-dependent carbohydrate-recognition proteins belonging to the innate immune response and bind glycans on the filovirus MLR in a GP-dependent fashion. The hepatocyte asialoglycoprotein receptor recognizes GPs with terminal galactose residues (Takada 2012). Other C-type lectins involved in filoviral infection are the dendritic cell and liver and lymph node-specific ICAM-3-grabbing non-integrin (DC/L-SIGN), the human macrophage galactose-type C-type lectin (hMGL), and the liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin). These molecules enhance viral infection, but do not appear to mediate attachment and viral fusion (Takada 2012). C-type lectins are expressed by all of the preferred human filovirus target cells, including hepatocytes, dendritic cells, and monocyte/macrophages. Their infection is linked to the diseases' hemorrhagic manifestation and immune disorders (Matsuno *et al*. 2010). The soluble form of mannose-binding C-type lectin is protective against ebolavirus infection. The ability to use DC-SIGN and hMGL to promote cellular entry correlates with filovirus pathogenicity, and the overall MLR amino acid sequence does not appear to be the primary factor in the differences (Takada 2012). Interestingly, viral carbohydrate binding affinity to host C-type lectins does not correlate with the differential efficiency of lectin-mediated entry by different MARV strains, although a critical amino acid at position 547 was found to be in close proximity to a cathepsin processing site (Matsuno *et al*. 2010).

Another host cell molecule known to be involved in cellular infection by MARV and EBOV is the ubiquitous human folate receptor-α, a glycosylphosphatidylinositol-linked surface protein. A pseudovirus containing EBOV GP could not infect T lymphocytes expressing this molecule, however, implying that it is not sufficient for cellular infection.

Finally, the endo/lysosomal cholesterol transporter protein NPC1, but not NPC2, is a ligand for the receptor-binding region of GP1 and is required for filovirus cellular infection (Miller *et al*. 2012; M. Ng *et al*. 2014). NPC1 is a large, hydrophobic, 13-pass transmembrane protein that is involved in endosome fusion with lysosomes and fission, calcium homeostasis, and HIV-1 release. It is highly conserved in animals, as is the NPC1-binding region of GP1among filoviruses (Miller *et al*. 2012). Disruption of the cholesterol transporting role of NPC1 is not required for its filovirus binding activity, nor is an acidified environment (Miller *et al*. 2012). NPC1 is also not involved in forming macropinosomes or in early internalization steps (Carette *et al*. 2012). Endosomal function, however, is important for filoviral entry of the cytoplasm since viral GP1 is cleaved by endosomal cathepsin, removing heavily glycosylated regions, exposing its receptor-binding region, allowing binding to the second luminal loop of NPC1, and mediating membrane fusion by viral GP2 (Miller *et al*. 2012; Takada 2012). NPC1 functions as an unusual filoviral receptor that recognizes its ligand within the endolysosome, rather than at the plasma membrane, by binding directly and specifically to the GP1 subdomain of the cleaved form of viral GP. Exposure of the NCP1 binding site in this intracellular location may be a mechanism by which filoviruses avoid interactions with host neutralizing antibodies (Miller *et al*. 2012).

Mutations in ebolaviruses GP alter virus host cell and species range via changes in the relative affinity of filoviral GP for NPC1 and the level of NPC1 on the cells (Martinez *et al*. 2013). While cultured cells from Büttikofer's epauletted fruit bats (*Epomops buettikoferi*) and the Egyptian rousette (*Rousettus aegypticus*) are susceptible to infection by ebolaviruses, cultured fibroblast, kidney, and lung cells from African straw-colored fruit bats (*Eidolon helvum*) have a greatly reduced susceptibility (Ng *et al*. 2015). Cell lines from all of these bat species are, however, susceptible to infection by MARV. The difference in ebolavirus tropism may be traced to a single amino acid change in the NPC1 of an *E. helvum* fibroblast cell line, which also decreases its affinity for interactions with the viral GP receptor in pseudotyped viruses bearing EBOV GP, and to a lesser extent, pseudotyped viruses expressing GP from Bundibugyo virus and Côte d'Ivoire ebolavirus of humans. When *E. helvum* cells were engineered to express human NPC1, they had a significantly increased susceptibility to EBOV infection. Comparative analysis of *NPC1* sequences from *E. helvum* cells and those from six other bat species (two non-African pteropodid, two phyllostomid, and two vespertilionid bats) strongly supported the presence of positive selection in one particular bat *NPC1* codon at position 502, the same position implicated in susceptibility or resistance to ebolavirus infection above (Ng *et al*. 2015). A viral GP V141A variant, found in LLOV and SEBOV, appears to also negatively influence viral entry into host cells. Pseudoviruses bearing the V141A mutation had a reduced ability to infect both *E. helvum* and *R. aegypticus* bat fibroblasts. Taken together, these data strongly support critical residues of host cell NPC1 and viral GP as being critical determinants of ebolavirus susceptibility in bats and demonstrates that variations in these molecules may represent host or viral adaptations to reduce infection by certain filoviruses.

TIM-1 and NPC1 have been reported to co-localize and interact in the endolysosomes at the sites of viral fusion. TIM-1 may function as a bridge that brings the cleaved GP to the vicinity of NPC1, permitting viral fusion with the endolysosomal membrane. Antibodies that disrupt the interaction between TIM-1 and NPC1 prevent viral fusion (Kuroda *et al*. 2015).

Filoviruses also circumvent the immune system by utilizing virus-specific antibodies for cellular infection in a mechanism known as antibody-dependent enhancement. Anti-GP antibodies bind to one of the cellular Fc receptors or ligands of the innate immune complement component C1q. Fc receptors are expressed exclusively on some cells of the immune system, including monocyte/macrophages, while C1q ligands are present on most mammalian cells. The viral components recognized by both the infection-enhancing GP-specific antibodies and neutralizing antibodies to filoviruses are primarily found located in the MLR, but react with different regions (Takada 2012). Since the structure of the MLR is highly variable and there is little cross-reactivity in anti-filoviral sera, the antibody-dependent enhancement is virus species-specific and also correlates with pathogenicity (Takada 2012).

4.2 MARBURG VIRUS

4.2.1 Marburg virus in humans and bats

There has long been circumstantial evidence linking filovirus of primates with bat exposure. During the first known outbreak of MARV in primate facilities in Europe in 1967, the infected monkeys were caught on the shores of Lake Victoria and islands where fruit bats are abundant (reviewed by Towner *et al*. 2009). The second recorded outbreak in Zimbabwe, in 1979, involved tourists who slept in rooms containing insectivorous bats. They had previously visited Chinhoyi caves, where they may have also encountered bats. In 1980 and 1987, visitors to Kitum Cave, Kenya, became infected with MARV. The cave is used by both fruit and insectivorous bats and, in 2007, MARV RNA was detected in pooled liver, spleen, and lung samples from a clinically healthy, pregnant *R. aegyptiacus* from the cave (Kuzmin *et al*. 2010). This RNA was relatively distant from others previously found in Kenya (Musoke and Ravn) and was more similar to the Popp and Ci67 strains from European primate colonies in 1967.

A prolonged outbreak (1998–2000) of Marburg hemorrhagic fever in Durba, in northeastern Democratic Republic of the Congo (DRC), was fueled by multiple transmission events to miners. At least nine genetically distinct viruses were circulating in the affected human population during that single outbreak (reviewed in Pasweska *et al*. 2012). Almost all of the affected miners (94%) worked in the underground Goroumbwa Mine, and not in the seven above-ground mines in the area. The mine is home to at least 10 000 roosting *R. aegyptiacus* bats as well as substantial numbers of *Rhinolophus eloquens* and *Miniopterus inflatus* insectivorous bats. Between 3.0% and 3.6% *M. inflatus* ($n = 33$), *R. eloquens* ($n = 197$), and *R. aegyptiacus* ($n = 127$) bats had detectable MARV RNA in multiple tissues (Swanepoel *et al*. 2007). Additionally, anti-MARV antibodies were present in 9.7% and 20.5% of the insectivorous and fruit bats, respectively. Interestingly, a *syndrome hémorragique de Durba* (hemorrhagic syndrome of Durba) has been linked to the mine since at least

1987 (reviewed in Swanepoel *et al*. 2007). The 1998–2000 outbreak ended when the mine flooded, supporting the hypothesis that this cave was involved in MARV transmission to humans (reviewed by Towner *et al*. 2009).

In 2005–2006, MARV RNA was detected in less than 2% of *R. aegyptiacus* bats' homogenized liver and spleen sample using real-time PCR. These bats were trapped near caves at two sites in Gabon that are 250 km apart and about 700 km north of Uige, Angola, the location of a large human MARV outbreak in 2005 (Towner *et al*. 2007). The bat RNA differed by 5% from that isolated in Angola. Interestingly, 9% of these bats also had low levels of MARV-specific IgG in their serum, suggesting previous exposure to the virus. Unlike EBOV, no MARV RNA was found in *Micropteropus pusillus* ($n=149$), *Myonycteris torquata* ($n=264$), *Epomops franqueti* ($n=296$), or *Hypsignathus monstrosus* ($n=57$) in Gabon (Towner *et al*. 2007).

In 2007, MARV outbreaks occurred in the Kitaka Mine in Uganda in miners having close contact with bats, some of whom had detectable viral RNA. Two bat MARV isolates were similar to the historical Marburg virus sequences and are most closely related (99.3% identical) to the sequence from one miner, while three other bat isolates are of the Ravn lineage and are more closely related (99.2–99.9% identical) to the sequence from another miner (Towner *et al*. 2009). The mine housed large numbers of *R. aegyptiacus* and *Hipposideros* species bats. Live MARV was isolated from homogenized spleen and liver samples from four *R. aegyptiacus* in 2007 and from another bat from the same colony in 2008, demonstrating that colonies may be infected for at least 9 months. Of the *R. aegyptiacus* bats tested on two occasions, 5.1% ($n=611$) were positive for MARV RNA, while only 0.2% ($n=609$) of the *Hipposideros* species animals had detectable RNA, perhaps indicating a spill-over event between these bat groups. While some of the *R. aegyptiacus* bats contained low titers of MARV-specific antibodies, none of the tested *Hipposideros* species bats did (Towner *et al*. 2009). Detection of RNA in older, mostly weaned, juveniles exceeds that of adults in general and pregnant females (10.3, 4.2, and 2.1%, respectively). Young juveniles caught during breeding seasons would be barely independent and recently released from the physically close protection of their mother and from receiving IgA antibodies from milk (Amman *et al*. 2012). Significantly, all placentas tested RNA-negative, suggesting that vertical transmission is not involved. Additionally, no infected pups were found in studies conducted in the mine or the cave although pups from antibody-positive mothers also had antibodies. Of note, no viral RNA was detected in oral swabs from bats, even those RNA detectable in their liver and spleen samples.

In 2007 and 2008, two tourists were infected with MARV after exposure to *R. aegyptiacus* bats in Python Cave, 30 miles from Kitaka Mine (reviewed in Towner *et al*. 2009). The bats at the cave and mine appear to be part of a metapopulation, since two animals tagged in the mine later were found in Python Cave, which houses in excess of 40 000 bats. A 2008–2009 study in Python Cave found that 2.5% of these bats had MARV RNA in multiple tissues. Multiple MARV RNA sequences from these bats closely matched that of one of the infected tourists (Amman *et al*. 2012), as well as bats from distant regions of Sub-Saharan Africa, such as Gabon and Zimbabwe. In South Africa, *R. aegyptiacus* bats move up to 32 km between roosting sites, and a marked female was even found to have relocated to a site 500 km away. This suggests that the bat movement over long distances may lead to exchange of viruses through a network of colonies throughout central and southern Africa (Amman *et al*. 2012).

A 2012 MARV outbreak in humans corresponded with a peak of MARV infections in bats. The full length genome sequences were almost identical to that of MARV from bats captured in 2008 and 2009 in a nearby cave. Filoviruses appear to undergo little genetic evolution during human-to-human transmission, the variation being believed to occur in the bat host population (Rodriguez *et al*. 1999; Towner *et al*. 2006).

4.2.2 Experimental infection of bats with Marburg virus

When juvenile *R. aegyptiacus* bats were inoculated subcutaneously with MARV or any of the five ebolavirus species, none of the animals demonstrated clinical symptoms, behavioral changes, or mortality (Amman *et al*. 2015; Jones *et al*. 2015). While viremia was present in all bats receiving MARV, none of the animals inoculated with ebolaviruses had detectable viral RNA in their blood. Paweska *et al*. (2012) had previously infected bats subcutaneously, but not by the nasopharyngeal route. In the MARV-positive bats infected subcutaneously, mean viral load value peaked on day 5 post-inoculation and was undetectable by day 10. The average time period of detectable viremia in bats was only 3 days (range = 1–9 days) (Amman *et al*. 2015). MARV RNA was found at multiple tissue sites in the euthanized bats: highest in the skin at the inoculation site, liver, and spleen (consistently high levels of RNA), but also in the salivary glands, testes, ovary/uterus, axillary and mesenteric lymph nodes, urinary bladder, small intestine, large intestine, heart, and kidney (Swanepoel *et al*. 2007; Amman *et al*. 2012, 2015; Paweska *et al*. 2012; Jones *et al*. 2015). In the organs potentially involved in viral shedding, the kinetics of infection differed from that of the blood: kidneys peaked on day 7 and were cleared by day 28, salivary glands and the large intestine peaked on day 8 and were cleared by day 12, and the bladder peaked on day 5 and was cleared by day 9 (Amman *et al*. 2015). It should be noted that, in addition to human-to-human transmission via blood, MARV can be spread among humans via semen.

In this study, MARV RNA was detectable on oral swabs on days 4–14 post-inoculation, with viral load peaking on day 8 (Amman *et al*. 2015). MARV was detectable for up to six consecutive days in these secretions. Of note, virus was present in some cases for 4 days after clearance from the blood. MARV transmission to other animals may be via the oral secretory route since fruit bats often test-bite fruit and produce large amounts of masticated fruit spats under fruit trees. Rectal samples also had low levels of virus, suggesting potential cross-species transmission by this route as well or via urine (technical difficulties prevented its collection in this study). Human contact with bat blood during preparation of bat meat for consumption or eating fruit contaminated with blood, urine, feces or placentas may also lead to zoonotic transmission (Paweska *et al*. 2012).

Viral RNA tissue distribution was much more limited and at lower levels in the ebolavirus-inoculated bats: for SUDV, in the skin of the inoculation site, liver, spleen, axillary lymph node, and urinary bladder; for EBOV, BOBEV, and REBOV, in the skin of the inoculation site and axillary lymph node; and for CIEBOV, only the inoculation site. Additionally, while MARV RNA was present in oral and rectal swabs from MARV-inoculated bats at 10 days post-inoculation, none was detected in any of the ebolavirus-inoculated animals in the same study (Jones *et al*. 2015). It thus appears that *R. aegypticus* bats are refractory to ebolavirus infection and are unlikely to be a natural ebolavirus reservoir despite reports of ebolavirus-seropositive bats of this species.

4.3 EBOLA VIRUS

4.3.1 Ebola virus in humans and bats

The first known outbreak of Ebola hemorrhagic fever was in 1976 and was caused by SEBOV. The initial patients worked in a cotton factory in Sudan containing roosting bats as well as many rodents. Additionally, the only reported human infection with CIEBOV occurred in 1994 in a researcher in Côte d'Ivoire who was studying a group of dead chimpanzees. These animals were known to have fed in a wild fig tree together with fruit bats 2 weeks prior to their illness (reviewed in Towner *et al*. 2009). It is not known whether the bats in either the cotton factory or the fig were infected.

A large study of bats from Gabon and the Republic of the Congo conducted in 2003–2006 detected ZEBOV-specific IgG in 6.8% of *E. franqueti* ($n=117$), 23.5% of 17 *H. monstrosus* ($n=17$), and 6.9% of *M. torquata* ($n=58$). Virus was present in the liver and spleen (Leroy *et al*. 2005). Two human outbreaks occurred in the Republic of the Congo during this time, in October 2003 and in May 2005. Interestingly, a seroprevalance of 5% was seen through both epidemic and nonepidemic regions during these outbreaks. Seroprevalence decreased to 1% following the outbreaks, suggesting a linkage between human outbreaks and bat infection or bat exposure to EBOV. Since many adult animals and pregnant *H. monstrosus* females were seropositive, bat-to-bat viral transmission may occur by fighting or sexual contact (Pourrut *et al*. 2007).

Another piece of evidence links bats and filoviruses over extended periods of time. Non-retroviral integrated RNA viruses are found within the genomes of many higher forms of life and serve as "living fossils." Bats contain copies of such integrated viruses that are homologous to two of the filovirus open reading frames. One of these insertions is believed to have been present in *Myotis* bats in similar genomic locations for 13.4 million years, while the other filovirus-like open reading frame predates the common ancestor of *Eptesicus* and *Myotis*, estimated at 25 million years. These insertions into bat genomes highlight the long relationship between bats and filoviruses (Taylor *et al*. 2011).

4.3.2 Ebola virus and bats prior to the 2014 outbreak

After the 2001–2003 ZEBOV outbreaks in Gabon and the Republic of the Congo, EBOV-specific antibodies were found in 8% of *H. monstrosus, E. franqueti*, and *M. torquata* bats and EBOV RNA in 5% of pooled liver and spleen samples (Leroy *et al*. 2005). A 2003–2008 study in these two countries found EBOV-specific IgG to be present in the following six bat species: *M. pusillus* (2%), *M. torquata* (3%), *E. franqueti* (4% positive), *H. monstrosus* (7%), *R. aegyptiacus* (8%), and *Mops condylurus* (12%). MARV-specific IgG was also present in *R. aegyptiacus* (7%), *H. monstrosus* (1%), and *M. pusillus* and *E. franqueti* (less than 1% of each). While no significant age- or gender-related difference was found in seroprevalence for either filovirus, yearly variation was seen, as well as higher incidences of EBOV-specific IgG in pregnant versus other female bats, similar to the higher prevalence of Hendra virus in pregnant *Pteropus scapulatus* females in Australia (Plowright *et al*. 2008; Pourrut *et al*. 2009). The percentage of MARV, but not EBOV, IgG-positive *R. aegyptiacus* appears to be greatest in those captured inside caves (14% versus 4% elsewhere). Gabon was, at least at that time, the only

country where bats exposed to both EBOV and MARV were reported, with IgG to both viruses only in *R. aegyptiacus* (Pourrut *et al*. 2009). IgG was present in many animals from which RNA was undetectable. Of note, while MARV RNA and infectious virus has been isolated from bats, no infectious ebolavirus has yet been isolated from bats and only one study detected ebolavirus RNA in bats (Leroy *et al*. 2005; Jones *et al*. 2015).

Between May and November 2007 in the DRC, a large human EBOV outbreak occurred, causing over 260 cases and 186 deaths. Area residents reported no unusual morbidity or mortality among wild or domestic animals. It should be noted that chimpanzees and gorillas are not found in this part of the DRC. An unusually massive annual fruit bat migration occurred that spring and migrating animals settled in the outbreak area between April and May, nestling in the numerous fruit trees and in palm trees of a neglected plantation. Area villagers hunted and killed large numbers of the bats using machetes, catapults, shotguns, or by hand, with thousands of human exposures to bat blood occurring. The bats serve as a major protein source for residents, specifically men, postmenopausal women, and children since women of child-bearing age are not permitted to eat bats, but will butcher, prepare, and cook them. In May, the putative index case had prepared freshly killed bats from hunters to eat, supporting a linkage between this Ebola outbreak and exposure to fruit bats. These findings also suggest that the massive seasonal fruit bat migrations should be considered when assessing Ebola risk predictions (Leroy *et al*. 2009). It may, however, prove to be difficult to stop area residents from consuming fruit bats, since they are a readily available and abundant source of protein in areas where many game animals are protected or becoming rare (Leroy *et al*. 2009).

In 2008–2009, REBOV-specific IgG was detected in 31% of tested *Rousettus amplexicaudatus* bats in the Philippines. No viral RNA was amplified from these animals' spleens by PCR. The animals were taken from forests 30 and 60 km from a farm and primate colony where infected monkeys and pigs had been found (Taniguchi *et al*. 2011).

4.3.3 EBOV incidence in bats during and after the 2014 outbreak

The largest ebolavirus outbreak began in 2014 in West Africa and is believed to have originated from a single spillover from the insectivorous bat *M. condylurus* to a young child in Guinea. There was no evidence of a concurrent or recent outbreak in large wildlife, including chimpanzees, which are highly susceptible to fatal infection. While exposure to fruit bats is common in the region due to their use as a food source, the index case is believed to have been infected by playing in a hollow tree housing a colony of *M. condylurus* (Saéz *et al*. 2015). After a fire burnt down this tree, many of its bat residents were captured by villagers who did not eat them due to a ban imposed the following day. Many villagers were exposed to the bat colony believed to have initiated the outbreak, however, without becoming infected. Insectivorous bats in the area also commonly roost under the roofs of houses and in similar hides in villages.They are often hunted by children and grilled over small fires. Despite the fact that *M. condylurus, E. helvum*, and *H. monstrosus* have previously been found to be seropositive for ebolaviruses, no EBOV RNA was detected in bats of these species in the region during the outbreak (Saéz *et al*. 2015).

The outbreak spread to several regional countries. Large endogenous chains of infection occurred in nearby Sierra Leone and Liberia, with smaller chains of transmission also present in Nigeria. Additionally, several cases were imported into other regions of the world, including Europe and the US, by people coming into the country from

West Africa, often aid workers or patients brought into the countries for treatment. The outbreak in Africa was sustained by multiple rounds of human-to-human transmission, often by contact with bodily fluids during burial preparations, at funerals, or in medical facilities. Many healthcare providers were stricken and died. The filovirus responsible for this outbreak was a new strain of EBOV, but the geographical range and the very large chains of human transmission were unique to this outbreak as was its purported association with insectivorous bats. Fortunately, the fatality rate was lower in this outbreak than occurred in some of those occurring previously, 30–40% as compared with rates in some outbreaks approaching 90%. The virus itself was very similar to EBOV isolates in central Africa.

4.4 LLOVIU AND RELATED FILOVIRUSES IN BATS

In contrast to the well-characterized ebolaviruses and MARV of Africa and Asia, Lloviu virus has been found only in a European bat. Its RNA was reported in the lungs, livers, rectal swabs, and spleens of *M. schreibersii* bat carcasses in Cueva del Lloviu, Spain in 2011 (Negredo *et al*. 2011). The virus has not been seen in other animal groups.

A study of nucleic acids from healthy *R. leschenaultia* bats in China revealed sequences related to filoviruses. Phylogenetic analysis of the Bt-DH04 filovirus strain places it with LLOV at a basal position situated between EBOV and MARV. Its *F1* gene has nucleotide identities of 46–49% to ebolaviruses, 44% to LLOV, and less than 40% to MARV (He *et al*. 2015).

4.5 SEASONALITY OF FILOVIRUS INFECTION IN BATS

Intraspecies infection in bats may involve biting, either during altercations in crowded colonies or by males biting females unwilling to mate, or licking that occurs during the mating process (Amman *et al*. 2015). Arthropod parasites of bats have thus far not been found to host MARV (Amman *et al*. 2012). Low levels of horizontal transmission appear to continue throughout the year with pulses of infection occurring in older juveniles that coincides with two annual birthing seasons (Amman *et al*. 2012). In Python Cave (which is actually more of a tunnel), the numerous nooks, crevices, and hidden chambers are heavily utilized by roosting bats, with most juvenile bats roosting in areas near the cave openings on the ground, inside and outside the tunnel proper, while adults occupy the darker interior spaces (Amman *et al*. 2012). *R. aegyptiacus* become pregnant around November and May and give birth in February and August, respectively. Interestingly, records of probable spill-over events into humans indicate that 83% of these events occurred during the above seasonal pulses (Amman *et al*. 2012). Hayman (2015) developed a model that posits that bi-annual breeding of bats and longer incubation periods are important for filovirus persistence in colony sizes consistent with those found naturally. The validity of this model is reinforced by serological data which show that bats from species with two annual birth pulses are more than four times more likely to be infected than those with a single annual birthing period.

In EBOV, the two birthing periods of the potential ebolavirus reservoir bat species (*E. franqueti, H. monstrosus*, and *M. torquata*) occur during the dry seasons when fruit

is scarce in the forest and the great ape and bat populations compete for fruit, allowing closer and more frequent interspecies contacts (Pourrut *et al*. 2007). In these bats, the rate of seroprevalence is higher in adults, particularly pregnant females, than in juveniles.

4.6 FACTORS AFFECTING ZOONOTIC INFECTION BY FILOVIRUSES

Pigott *et al*. (2014) has produced an excellent set of predictive mapping distributions of human, zoonotic, and bat niches for African ebolavirus infections. They utilized *H. monstrosus, M. torquataI*, and *E. franqueti* bats as the most likely reservoir species and utilized the Global Biodiversity Information Facility as well as expert opinion maps of the known bat species ranges in their work. Marginal effect plots for these bat species were strongly affected by land surface temperature as well as vegetation. Walsh and Haseeb (2015) also found that vegetation density is an important factor in zoonotic spillover of Ebola to humans. An inverse relationship exists between spillover and both temperature and altitude while a positive relationship is found with higher absolute humidity (Walsh & Haseeb 2015; S. Ng *et al*. 2014). Interestingly, rates of seropositivity may be linked to fighting and mating behaviors, shown to be most frequent during rainy or wet seasons. Since ebolaviruses persist in infective form for months in human semen, similar findings may also be true in the bat hosts (S. Ng *et al*. 2014).

Several serological studies conducted in Zambia and Ghana detected EBOV-specific IgG in migratory *E. helvum* (Hayman *et al*. 2010; Ogawa *et al*. 2015). Finding EBOV-specific antibodies in this tree-roosting migratory fruit bat may be of importance since this species is common and found across Sub-Saharan Africa, living in colonies of up to several million animals, often in cities (Hayman *et al*. 2010). Since seroprevalence in these colonies is quite low (1 of 262 tested bats), this study indicates the exposure of these bat colonies to EBOV, although not necessarily infection. As expected, of the *E. helvum* collected in Zambia, most of the antibodies were specific to African filoviruses, such as ZEBOV, however, some of the sera contained IgG-specific for REBOV, previously found only in Asia, perhaps due to antibody cross-reactivity or to the actual presence of REBOV in Africa (Ogawa *et al*. 2015). Even though seroprevalence is less than 0.5% in tested bats, this study indicates that very large bat colonies are at least exposed to EBOV. This species is migratory and has the propensity to dwell in cities which could lead to dispersion of EBOV over long distances to new geographical regions in Africa, possibly in densely populated urban centers.

In Bangladesh, during the breeding season, antibodies to EBOV were detected by ELISA and Western blotting in 3.5% of *R. leschenaultii* bats, all of which were male. *Cynopterus* species bats and *Megaderma lyra* bats were also seropositive but only by ELISA. Interestingly, Western blot analysis found that almost all of the samples reacted more strongly to EBOV than to antigens from REBOV, the only species of filovirus known to be present in that region of the world. All tested throat, urine, urogenital, and fecal samples were PCR-negative for filovirus RNA (Olival *et al*. 2013). These studies suggest that while some species of ebolavirus may be much more widely distributed than had been thought, they may not play a significant role in spillover into humans due to low prevalence rates and the inability to demonstrate virus in the bats.

The bat species with the highest likelihood of zoonotic infection have a predicted distribution that extends throughout West and Central Africa, especially the rainforests of the northeastern, western and central Congo basin; Guinea; and coastal forest regions of the Congo (Pigott *et al*. 2014). Over 22 million people live in areas predicted to be suited for zoonotic transmission, with most living in rural areas. Those at highest risk are in DRC, Guinea, and Uganda, with people in Nigeria, Cameroon, and the Central African Republic at somewhat lesser risk. Interestingly, Liberia and Sierra Leon, two of the sites of the largest EBOV outbreak, were not indicated to be areas of high risk.

4.7 FILOVIRUSES IN ANIMALS OTHER THAN BATS

Seven human ebolavirus outbreaks (Côte d'Ivoire, 1994; Mekouka, 1994; Mayibout, 1996; Booué, 1996; Mekambo, 2001; Kelle, 2003; and Mbomo, 2003) have been clearly linked to exposure to dead chimpanzees, gorillas, monkeys, or duikers (Leroy *et al*. 2009). In an attempt to determine the filoviral reservoir for primate and duiker infections, a large number of plant (groundnut, beetroot, goosefoot weed, cucumber, pumpkin, soybean, *Gomphrena globosa*, cotton, lupin, tomato, siratro bean, wild tobacco, tobacco, French bean, green pea, wheat, broadbean, cowpea, and maize) and animal (domestic pigeon, painted reed frog, common toad, grey tree frog, tropical house gecko, brown house snake, leopard tortoise, hinged-back tortoise, *Tadarida condylura* – Angola free-tailed bat, *Tadarida pumila* – little free-tailed bat, *Epomophorus wahlbergi* – Wahlberg's epauletted fruit bat, multimammate mouse, NIH mouse, American cockroach, leafhopper, Myrmicine ant, social spider, millipede, and American land snail) species were experimentally inoculated with ZEBOV (Swanepoel *et al*. 1996). Significant viral titers were detected in the sera or pooled viscera samples from all three bat species in Vero cells using a fluorescent focusing assay. Virus was also recovered from the feces of an *E. wahlbergi* bat 21 days post-inoculation.

EBOV does not appear to be transmitted to humans via air or water, but may occur by handling bushmeat (wild animals hunted for food), including infected bats, or close contact with infected animals, such as chimpanzees, bats, and duikers. Between humans, filoviruses are transmitted by contact with blood, bodily fluids (including semen), tissues, organs, or skin of infected patients or corpses, including during funeral rituals (Rewar & Mirdha 2014). Many of the outbreaks have been linked to nosocomial transmission and healthcare workers are at high risk of infection.

REBOV is nonpathogenic in humans but causes severe hemorrhagic fever in macaques. Several REBOV epizootics have been reported in cynomolgus macaques in 1989, 1990, 1992, and 1996 and an outbreak in pigs in 2008 (Taniguchi *et al*. 2011). Additionally, several outbreaks have occurred in primate facilities in the US and Italy among monkeys imported from the Philippines. REBOV infection was also found in domestic pigs and pig workers in the Philippines. While it is unclear whether REBOV causes disease in swine, EBOV causes severe respiratory disease in experimentally infected animals (Takada 2012). In Africa, wild gorillas and chimpanzees have been infected with EBOV and developed severe to fatal disease, and viral RNA has also been found in duikers, relatives of antelopes and gazelles. EBOV-seropositive dogs have also been reported, presumably exposed by eating infected dead animals or licking infected humans (Takada 2012).

Mice and guinea pigs experimentally infected with filoviruses derived from patients permit viral replication but generally do not develop fatal disease. Serial passage of ebolaviruses and MARV through guinea pigs, does lead to increased lethality, however, and passage of EBOV through young mice selects for highly lethal mouse viral variants (Takada 2012). Alteration of GP is not the driving factor for this increase in lethality.

In the Philippines, oropharyngeal swabs from *M. schreibersii* bats contained low levels of REBOV RNA that differed by a single nucleotide from a pig isolate. Pooled urine and rectal swabs were negative (Jayme *et al*. 2015). Additionally, antibodies were detected by ELISA and Western blotting in *Acerodon jubatus* and by ELISA only in *Pteropus vampyrus*.

4.8 CONCLUSIONS

It is well documented that ebolavirus and MARV proteins impair type I and II IFN responses. Human nonsurvivors of filovirus infections also produce a highly proinflammatory milieu that includes hypersecretion of proinflammatory cytokines (IL-1β, IL-1RA, IL-6, IL-8, IL-15, and IL-16), as well as various chemokines and growth factors (MIP-1α, MIP-1β, MCP-1, M-CSF, MIF, IP-10, GRO-α, and eotaxin) that draw leukocytes into the affected area. Massive T cell apoptosis is also present in human nonsurvivors (Wauquier *et al*. 2010). A similar loss has not been reported in bats. Bat immunity to viruses relies primarily upon type I and type III IFNs in the absence of a detrimental proinflammatory response. The effects of filoviruses upon inflammatory reactions to filoviruses in bats as well as upon type III IFNs have not been reported, suggesting that bats may well be protected by the latter anti-viral immune mediators in the absence of an inflammatory response. These responses to filovirus infection of bats may at least partially explain the different effects of ebolavirus and MARV in humans and bats. Other possible differences in human and bat reactions to filovirus infections exist. Experimental infection of various bat cell lines leads to accumulation of large numbers of viral particles in the cells in contrast to viral release in filovirus-infected human cells. Antibody-dependent enhancement occurs in infected humans as well, but this reaction has not reported in bats.

Viral binding proteins as well as host receptors are critical components for host species and host cell tropism. Human cell surface TIM-I, Tyro3 receptor tyrosine kinase family members, DC/L-SIGN, hMGL, and LSECtin and the endosomal NPC1 serve as receptors for ebolavirus attachment and entry into human target cells, but their bat homologs may or may not serve this function in bat cells. Cultured cells from several bats (*E. buettikoferi, R. aegyptiacus*, and *E. helvum*), nevertheless, can be infected by MARV and ebolaviruses, however *E. helvum* is much less susceptible to ebolavirus infection, perhaps due to a single amino acid change in its NPC1.

Several chains of circumstantial evidence link Marburg infection in humans and nonhuman primates to bat exposure. The first known cases of MARV occurred in primate facilities which imported infected monkeys captured on islands in southern Africa that have abundant numbers of fruit bats. It is not known whether the bats from these islands were infected. MARV was subsequently found in tourists who visited various caves or mines in southern or Central Africa which housed several species of frugivorous and insectivorous bats. Even though these caves are major tourist destinations, very

few visitors have been infected with MARV, suggesting that any zoonotic transfer occurring in these caves is relatively rare in this human population. A higher incidence of MARV infection, however, has been seen among workers from several underground mines during a number of human outbreaks in the DRC, Gabon, and Uganda. Some of these mines contain large numbers of a variety of bat species. Some outbreaks appear to have resulted from multiple spill-over events and were temporally correlated with outbreaks in bats. Multiple tissues from frugivorous and insectivorous bats in these mines were found to be RNA-positive for one of several MARV strains. Additionally, full length MARV genomic sequences from virus derived from infected people during an outbreak were almost identical to those isolated earlier from bats in a nearby cave. The linkage between MARV in miners and bats, therefore, is much stronger than that in tourists. Further work is needed to determine whether bats are a major reservoir host or whether other cave inhabitants are also infected. The route of human transmission also needs to be established in order to avoid further MARV outbreaks.

Following experimental infection of *R. aegyptiacus* with MARV, viral RNA was isolated from multiple tissues, including those potentially involved in viral shedding, such as the kidneys, urinary bladder, salivary glands, and the large intestine, as well as blood. *R. aegypticus* appears to be less susceptible to experimental ebolavirus infection and has more limited tissue distribution, with some ebolavirus species found only at the injection site and the axillary lymph nodes. This particular bat species appears, therefore, to be less likely to serve as a reservoir host or an agent of zoonotic transmission of ebolaviruses.

Anti-ZEBOV IgG was found in several species of African bats between 2003 and 2006. Virus was also detected in some of the bats' spleens and livers. Yearly variance in viral seroprevalence is seen and, as is the case for several other viral groups, is higher in pregnant females, perhaps due to their decreased immune status. It should be noted that while no infectious ebolavirus has been isolated from seropositive or RNA-positive bats, a large EBOV outbreak temporally correlated with an unusually large fruit migration in the DRC. Many of these bats were used as bushmeat, thus exposing many area residents to bat blood. It is not known whether this population of bats was infected. Additionally, neither the factors underlying this massive bat migration nor the presence of similar increases in population size of other groups of animals, such as rodents, were reported.

A third group of filoviruses, with its sole species, Lloviu virus, has only been found in European bats. While it may not infect humans, this virus is of potential concern to bats since its RNA has been detected in multiple tissues in bat carcasses. This filovirus is unusual in its location and may cause pathology or death in other European bat populations. Nucleic acids from a distantly related filovirus were also found in a healthy Chinese bat.

More work is required in order to more accurately determine the threat of zoonotic infection of filoviruses into human populations. While circumstantial, a substantial chain of evidence implicates bats as at least one of the reservoir hosts of MARV linked to infection of humans with long-term intensive exposure to bats and their secretions in mines. Additional studies focusing on the route of human infection could aid in developing methods to reduce risk to the miners as well as to determine whether bats are the only or major MARV reservoir host. The linkage between ebolaviruses in bats and zoonotic transmission is less conclusive. Many of the studies report seropositivity or viral

RNA from experimentally infected animals, but have yet to isolate infectious virus from bats. Since several species of nonhuman primates and duikers are known to harbor infectious ebolaviruses, it may be profitable to focus attention on these species in order to determine how they become infected.

REFERENCES

Amman BR, Carroll SA, Reed ZD, Sealy TK, Balinandi S, Swanepoel R, Kemp A, Erickson BA, Comer JA, Campbell S, Cannon DL, Khristova ML, Atimnedi P, Paddock CD, Crockett RJK, Flietstra TD, Warfield KL, Unfer R, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Rollin PE, Ksiazek TG, Nichol ST, Towner JS. 2012. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. *PLoS Pathology.* 8(10): e1002877.

Amman BR, Jones MEB, Sealy TK, Uebelhoer LS, Schuh AJ, Bird BH, Coleman-McCray JD, Martin BE, Nichol ST, Towner JS. 2015. Oral shedding of Marburg virus in experimentally infected Egyptian fruit bats (*Rousettus aegyptiacus*). *Journal of Wildlife Diseases.* 51(1): 113–124.

Basler CF, Amarasinghe GK. 2009. Evasion of interferon responses by Ebola and Marburg viruses. *Journal of Interferon & Cytokine Research*. 29(9):511–520.

Beltz LA. 2011. Marburg and Ebola hemorrhagic fevers. In: *Foundations of Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey- Bass and APHA Press: San Francisco, CA, pp. 247–271.

Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, Cin PD, Dye JM, Whelan SP, Chandran K, Brummelkamp TR. 2012. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature.* 477(7364):340–343.

Hayman DT. 2015. Biannual birth pulses allow filoviruses to persist in bat populations. *Proceedings of Biological Science.* 282(1803):20142591.

Hayman DTS, Emmerich P, Yu M, Wang L-F, Suu-Ire R, Fooks AR, Cunningham AA, Wood JLN. 2010. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS ONE.* 5(8): e11978.

He B, Feng Y, Zhang H, Xu L, Yang W, Zhang Y, Li X, Tu C. 2015. Filovirus RNA in fruit bats, China. *Emerging Infectious Diseases.* 21(9):1675–1677.

Jayme SI, Field EH, de Jong C, Olival KJ, Marsh G, Tagtag AM, Hughes T, Bucad AC, Barr J, Azul RR, Retes LM, Foord A, Yu M, Cruz MS, Santos IJ, Lim TMS, Benigno CC, Epstein JH, Wang F-L, Daszak P, Newman SH. 2015. Molecular evidence of Ebola Reston virus infection in Philippine bats. *Virology Journal.* 12:107.

Jones MEB, Schuh AJ, Amman BR, Sealy TK, Zaki SR, Nichol ST, Towner JS. 2015. Experimental inoculation of Egyptian rousette bats (*Rousettus aegyptiacus*) with viruses of the *Ebolavirus* and *Marburgvirus* genera. *Viruses.* 7:3420–3442.

Krähling V, Dolnik O, Kolesnikova L, Schmidt-Chanasit J, Jordan I, Sandig V, Günther S, Becker S. 2010. Establishment of fruit bat cells (*Rousettus aegyptiacus*) as a model system for the investigation of filoviral infection. *PLoS Neglected Tropical Diseases.* 4(8): e802.

Kuroda M, Fujikura D, Nanbo A, Marzi A, Noyori O, Kajihara M, Maruyama J, Matsuno K, Miyamoto H, Yoshida R, Feldmann H, Takada A. 2015. Interaction between TIM-1 and NPC1 is important for cellular entry of Ebola virus. *Journal of Virology.* 89(12):6481–6483.

Kuzmin I, Niezgoda M, Franka R, Agwanda B, Markotter W, Breiman RF, Shieh W-J, Zaki SR, Rupprecht CE. 2010. Marburg virus in fruit bat, *Kenya. Emerging Infectious Diseases.* 16:352–354.

Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez J-P, Muyembe-Tamfum J-J, Formenty P. 2009. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector-Borne and Zoonotic Diseases.* 9(6):723–728.

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska JT, Gonzalez JP, Swanepoel R. 2005. Fruit bats as reservoirs of Ebola virus. *Nature.* 438:575–576.

Licata JM, Simpson-Holley M, Wright NT, Han Z, Paragas J, Harty RN. 2003. Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: involvement of host proteins TSG101 and VPS-4. *Journal of Virology*. 77:1812–1819.

Martinez O, Ndungo E, Tantral L, Miller EH, Leung LW, Chandran K, Basler CF. 2013. A mutation in the Ebola virus envelope glycoprotein restricts viral entry in a host species- and cell-type-specific manner. *Journal of Virology.* 87(6):3324–3334.

Maruyama J, Miyamoto H, Kajihara M, Ogawa H, Maeda K, Sakoda Y, Yoshida R, Takada A. 2014. Characterization of the envelope glycoprotein of a novel filovirus, Lloviu virus. *Journal of Virology.* 88(1):99–109.

Matsuno K, Kishida N, Usami K, Igarashi M, Yoshida R, Nakayama E, Shimojima M, Feldmann H, Irimura T, Kawaoka Y, Takada A. 2010. Different potential of C-type lectin-mediated entry between Marburg virus strains. *Journal of Virology.* 84(10):5140–5147.

Miller EH, Obernosterer G, Raaben M, Herbert AS, Deffieu MS, Krishnan A, Ndungo E, Sandesara RG, Carette JE, Kuehne AI, Ruthel G, Pfeffer SR, Dye JM, Whelan SP, Brummelkamp TR, Chandran K. 2012. Ebola virus entry requires the host-programmed recognition of an intracellular receptor. *The EMBO Journal*. 31:1947–1960.

Mylne A, Brady OJ, Huang Z, Pigott DM, Golding N, Kraemer MUG, Hay SI. 2014. A comprehensive database of the geographic spread of past human Ebola outbreaks. *Scientific Data* 1:140042.

Negredo A, Palacios G, Vázquez-Morón S, González F, Dopazo H, Molero F, Juste J, Quetglas J, Savji N, de la Cruz Martínez M, Herrera JE, Pizarro M, Hutchison SK, Echevarría JE, Lipkin WI, Tenorio A. 2011. Discovery of an ebolavirus-like filovirus in Europe. *PLoS Pathology.* 7:e1002304.

Ng M, Ndungo E, Jangra RK, Cai Y, Postnikova E, Radoshitzky SR, Dye JM, de Arellano ER, Negredo A, Palacios G, Kuhn JH, Chandran K. 2014. Cell entry by a novel European filovirus requires host endosomal cysteine proteases and Niemann–Pick C1. *Virology.* 468–470:637–646.

Ng M, Ndungo E, Kaczmarek ME, Herbert AS, Binger T, Kuehne AI, Jangra RK, Hawkins JA, Gifford RJ, Biswas R, Demogines A, James RM, Yu M, Brummelkamp TJ, Drosten C, Wang L-F, Kuhn JH, Müller MA, Dye JM, Sawyer SL, Chandran K. 2015. Filovirus receptor NPC1 contributes to species-specific patterns of ebolavirus susceptibility in bats. *eLife.* 4:e11785.

Ng S, Basta NE, Cowling BJ. 2014. Association between temperature, humidity and ebolavirus disease outbreaks in Africa, 1976 to 2014. *European Surveillance*. 19(35):pii = 20892. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20892. Accessed May 8, 2017.

Ogawa H, Miyamoto H, Nakayama E, Yoshida R, Nakamura I, Sawa H, Ishii A, Thomas Y, Nakagawa E, Matsuno K, Kajihara M, Maruyama J, Nao N, Muramatsu M, Kuroda M, Simulundu E, Changula K, Hang'ombe B, Namangala B, Nambota A, Katampi J, Igarashi M, Ito K, Feldmann H, Sugimoto C, Moonga L, Mweene A, Takada A. 2015. Seroepidemiological prevalence of multiple species of filoviruses in fruit bats (*Eidolon helvum*) migrating in Africa. *Journal of Infectious Diseases*. Mar 18:pii:jiv063.

Olejnik J, Ryabchikova E, Corley RB, Muhlberg E. 2011. Intracellular events and cell fate in filovirus infection. *Viruses.* 3:1501–1531.

Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, Khan SU, Crameri G, Wang L-F, Lipkin WI, Luby SP, Daszak P. 2013. Ebola virus antibodies in fruit bats, Bangladesh. *Emerging Infectious Diseases.* 19(2):270–273.

Paweska JT, Jansen van Vuren P, Masumu J, Leman PA, Grobbelaar AA, Birkhead M, Clift S, Swanepoel R, Kemp A. 2012. Virological and serological findings in *Rousettus aegyptiacus* experimentally inoculated with Vero cells-adapted Hogan strain of Marburg virus. *PLoS ONE.* 7(9):e45479.

Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ, Brady OJ, Kraemer MU, Smith DL, Moyes CL, Bhatt S, Gething PW, Horby PW, Bogoch II, Brownstein JS, Mekaru SR, Tatem AJ, Khan K, Hay SI. 2014. Mapping the zoonotic niche of Ebola virus disease in Africa. *eLife*.3:e04395.

Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, Daszak P, Foley JE. 2008. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of Biological Sciences*. 275(1636):861–869.

Pourrut X, Délicat A, Rollin PE, Ksiazek TG, Gonzalez J-P, Leroy EM. 2007. Spatial and temporal patterns of *Zaire ebolavirus* antibody prevalence in the possible reservoir bat species. *The Journal of Infectious Diseases.* 196:S176–S183.

Pourrut X, Souris M, Towner JS, Rollin PE, Nichol ST, Gonzalez J-P, Leroy E. 2009. Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. *BMC Infectious Diseases.* 9:159.

Rewar S, Mirdha D. 2014. Transmission of Ebola virus disease: an overview. *Annals of Global Health.* 80:444–451.

Rodriguez LL, De Roo A, Guimard Y, Trappier SG, Sanchez A, Bressler D, Williams AJ, Rowe AK, Bertolli J, Khan AS, Ksiazek TG, Peters CJ, Nichol ST. 1999. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. *Journal of Infectious Diseases.* 179(Suppl. 1):S170 –S176.

Saéz AM, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Düx A, Kühl HS, Kaba M, Regnautn S, Merkel K, Sachse A, Thiesen U, Villányi L, Boesch C, Dabrowski PW, Radonić A, Nitsche A, Leendertz SAJ, Petterson S, Becker S, Krähling V, Couacy-Hymann E, Akoua-Koffi C, Weber N, Schaade L, Fahr J, Borchert M, Gogarten JF, Calvignac-Spencer S, Leendertz FH. 2015. Investigating the zoonotic origin of the West African Ebola epidemic. *EMBO Molecular Medicine.* 7:17–23.

Swanepoel, R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, Rollin PE, Zaki SR, Peters CJ. 1996. Experimental inoculation of plants and animals with Ebola virus. *Emerging Infectious Diseases.* 2:321–325.

Swanepoel R, Smit SB, Rollin PE, Formenty P, Leman PA, Kemp A, Burt FJ, Grobbelaar AA, Croft J, Bausch DG, Zeller H, Leirs H, Braack LEO, Libande ML, Zaki S, Nichol ST, Ksiazek TG, Paweska JT, on behalf of the International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo. 2007. Studies of reservoir hosts for Marburg virus. *Emerging Infectious Diseases.* 13(12):1847–1851.

Takada A. 2012. Filovirus tropism: cellular molecules for viral entry. *Frontiers in Microbiology*. 3:Article 34.

Taniguchi S, Watanabe S, Masangkay JS, Omatsu T, Ikegami T, Alviola P, Ueda N, Iha K, Fujii H, Ishii Y, Mizutani T, Fukushi S, Saijo M, Kurane I, Kyuwa S, Akashi H, Yoshikawa Y, Morikawa S. 2011. Reston Ebolavirus antibodies in bats, the Philippines. *Emerging Infectious Diseases.* 17(8):1558–1559.

Taylor DJ, Dittmar K, Ballinger MJ, Bruenn JA. 2011. Evolutionary maintenance of filovirus-like genes in bat genomes. *BMC Evolutionary Biology*. 11:336.

Timmins J, Schoehn G, Ricard-Blum S, Scianimanico S, Vernet T, Ruigrok RW, Weissenhorn W. 2003. Ebola virus matrix protein VP40 interaction with human cellular factors Tsg101 and Nedd4. *Journal of Molecular Biology*. 326:493–502.

Towner JS, Amman BR, Sealy TK, Carroll SAR, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PBH, Albarino CG, Miller DM, Reed ZD, Kayiwa JT, Mills JN, Cannon DL, Greer PW, Byaruhanga E, Farnon EC, Atimnedi P, Okware S, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Ksiazek TG, Nichol ST, Rollin PE. 2009. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathology.* 5(7):e1000536.

Towner JS, Khristova ML, Sealy TK, Vincent MJ, Erickson BR, Bawiec DA, Hartman AL, Comer JA, Zaki SR, Stroher U, Gomes DA Silva F, del Castillo F, Rollin PE, Ksiazek TG, Nichol ST. 2006. Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. *Journal of Virology.* 80:6497– 6516.

Towner JS, Pourrut X, Albariño CG, Nkogue CN, Bird BH, Grard G, Ksiazek TG, Gonzalez J-P, Nichol ST, Leroy EM. 2007. Marburg virus infection detected in a common African bat. *PLoS ONE*. 2(8): e764.

Urata S, Noda T, Kawaoka Y, Morikawa S, Yokosawa H, Yasuda J. 2007. Interaction of Tsg101 with Marburg virus VP40 depends on the PPPY motif, but not the PT/SAP motif as in the case of Ebola virus, and Tsg101 plays a critical role in the budding of Marburg virus-like particles induced by VP40, NP, and GP. *Journal of Virology*. 81:4895–4899.

Walsh MG, Haseeb MA. 2015. The landscape configuration of zoonotic transmission of Ebola virus disease in West and Central Africa: interaction between population density and vegetation cover. *PeerJ.* 3:e735.

Wauquier N, Becquart P, Padilla C, Baize S, Leroy EM. 2010. Human fatal Zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. *PLoS Neglected Tropical Diseases.* 4(10):e837.

5

BATS AND CORONAVIRUSES

5.1 INTRODUCTION

Many species of coronaviruses exist among humans and animals, including in bats, birds, cats, dogs, pigs, mice, livestock (horses, sheep, cattle, and camels), and whales, but no host-specific coronavirus (CoV) has been reported in monkeys or apes. Coronaviruses that have been reported to be associated with bats are found in Table 5.1. Coronaviruses cause mild to highly severe or fatal respiratory, enteric, hepatic, or neurological disease. The first two coronaviruses known to infect humans were HCoV-229E and HCoV-OC43, found in the 1960s to cause typically mild respiratory illnesses (reviewed in van Boheemen *et al*. 2012). Two other species, however, cause diseases with a high mortality rate in humans: severe acute respiratory syndrome coronavirus (SARS-CoV), discovered in 2003, and Middle East respiratory syndrome virus (MERS-CoV), found in 2012. The much less pathogenic HCoV-NL63 and HCoV-HKU1were characterized in 2004 and 2005, respectively (reviewed in van Boheemen *et al*. 2012).

Coronaviruses belong to the family Coronaviridae, subfamily Coronoavirinae of the order Nidovirales. There are four genera of coronaviruses – α, β, γ, and δ. Alpha- and betacoronaviruses have only been reported in mammals and members of both groups sicken humans to some extent. Coronaviruses are enveloped and spherical, with a ssRNA (+) genome. The genome is 27–32 kb and is the largest among that of all known RNA viruses. Its envelope is studded with spikes.

Evidence for exposure or infection with coronaviruses is present in eleven of the eighteen bat families from either frugivorous or insectivorous mega- and microbats and harbor alpha- or betacoronaviruses (reviewed by Drexler *et al*. 2014). The majority of

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TABLE 5.1 Coronaviruses associated with bats

Bat family	Bat common name	Bat species	Coronavirus species
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	Betacoronavirus sp.
Pteropodidae	Malagasy fruit bat	*Eidolon dupreanum*	Betacoronavirus sp.
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Alphacoronavirus sp.
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Betacoronavirus sp.
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Betacoronavirus, lineage c
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	ARCoV, alphacoronavirus
Rhinolophidae	Intermediate roundleaf bat	*Hipposideros larvatus*	Betacoronavirus sp.
Rhinolophidae	Pomona roundleaf bat	*Hipposideros pomona*	HKU10 alphacoronavirus
Rhinolophidae	Pomona roundleaf bat	*Hipposideros pomona*	HpBtCoV/3740-2
Vespertilionidae	Savi's pipistrelle	*Hypsugo savii*	2c, betacoronavirus
Vespertilionidae	Japanese long-fingered bat	*Miniopterus fuliginosus*	HKU1 alphacoronavirus
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	HKU8 alphacoronavirus
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	Alphacoronavirus sp.
Vespertilionidae	Pond bat	*Myotis dasycneme*	Alphacoronavirus sp.
Vespertilionidae	Cape bat	*Neoromicia capensis*	NeoCoV, MERS-like betacoronavirus
Vespertilionidae	Zulu serotine	*Neoromicia* cf. *zuluensis*	PML/2011, betacoronavirus
Vespertilionidae	Japanese pipistrelle	*Pipistrellus abramus*	HKU5, bat betacoronavirus, lineage c
Vespertilionidae	Kuhl's pipestrelle	*Pipiestrellus kuhlii*	Alphacoronavirus sp.
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	VM31, betacoronavirus
Pteropodidae	Madagascan flying fox	*Pteropus rufus*	Betacoronavirus sp.
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	LYRa11, SARS-related betacoronavirus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Rf1, SARS-like betacoronavirus, lineage b
Rhinolophidae	Great-eared horseshoe bat	*Rhinolophus macrotis*	Rm1, SARS-like betacoronavirus, lineage b
Rhinolophidae	Pearson's horseshoe bat	*Rhinolophus pearsonii*	SARS-like bat betacoronavirus
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus*	SARS-like bat betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	SARS-like bat betacoronavirus, lineage b
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	RsSHC014, bat betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Rs3367, clade 1 bat betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Rp3, clade 1 bat betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	HKU1 alphacoronavirus

(*Continued*)

TABLE 5.1 (Continued)

Bat family	Bat common name	Bat species	Coronavirus species
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	HKU2 alphacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	HKU8 alphacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	RaBtCoV/4991 SARS-like betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Rs806, clade 2 bat betacoronavirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Rs672, bat betacoronavirus
Pteropodidae	Flying foxes	*Rousettus* sp.	HKU9, bat betacoronavirus, lineage d
Vespertilionidae	Lesser bamboo bats	*Tylonycteris pachypus*	HKU4, bat betacoronavirus, lineage c
Vespertilionidae	Asian parti-colored bat	*Vespertilio superans*	SC2013, bat betacoronavirus

bat coronaviruses, however, have been reported in insectivorous bats and only four species in frugivorous bats. The straw-colored fruit bat (*Eidolon helvum*) is linked to one unclassified alpha- and one unclassified beta-CoV. Interestingly, only two of the four frugivorous bat species are infected by a SARS-like coronavirus: the Malagasy fruit bats (*Eidolon dupreanum*) and the Madagascan flying fox (*Pteropus rufus*) (Razanajatovo *et al*. 2015). Both of these bats are found only in Madagascar, while the SARS epidemic originated in China and is believed by many to have passed from Chinese fruit bats to civit cats and raccoon dogs before infecting humans. Of note, all bat species known to harbor SARS-like coronaviruses in Asia or Southeast Asia are from the insectivorous Rhinolophidae horseshoe bat family (*Rhinolophus ferrumequinum, R. macrotis, R. pearsonii, R. sinicus*, and *R. pusillus*) and not from fruit bats. The bats most closely associated with human MERS-CoV are also insectivorous, but are found in Africa and the Middle East, in regions where MERS is also present. Interestingly, SARS-CoV-like and MERS-CoV-like bat cornonaviruses have recently been reported in Korea (Kim *et al*. 2016). The authors mentioned that Korea experienced a MERS outbreak, however, since the index case had just travelled to the Middle East, it is not likely that bats pose a threat for zoonotic transmission to humans in Korea.

Infection of people by human coronaviruses HCoV-NL63, HCov-229E, HCoV-OC43 (originating in cattle), and HCoV-HKU1 are self-limiting, common cold-like illnesses, however, as is the case for most microbial infections, more severe symptoms may occur in children, the elderly, and immunocompromised patients. Alphacoronaviruses have a broader host range and genetic diversity than betacoronaviruses in bats and have been reported in Asia and Southeast Asia, North America, Africa, and Australia (Ge *et al*. 2013; Drexler *et al*. 2014). Betacoronaviruses have, however, been reported in bats from Thailand, the Philippines, Mexico, Neotropical South America, China, the Philippines, Madagascar, Kenya, South Africa, and the Middle East (reviewed by Drexler *et al*. 2014; Razanajatovo *et al*. 2015). HCoV-229E and HCoV-NL63 are alphacoronaviruses, while SARS- and MERS-CoV are betacoronaviruses. Betacoronaviruses are

divided into four lineages (lineages a–d). The human HCoV-OC43 and HCoV-HKU1 belong to lineage a: SARS-CoV, civet SARS-related coronaviruses, and SARS-related *Rhinolophus* bat coronaviruses belong to lineage b; and HCoV-EMC/2012 (EMC/2012) and MERS-CoV belong to lineage c. Both betacoronavirus lineages c and d include viruses detected in bats, such as HKU4 bat CoV from the lesser club-footed bat (*Tylonycteris pachypus*) and HKU5 bat CoV from the Japanese pipistrelle (*Pipistrellus abramus*) (both lineage c beta-CoV) and the *Rousettus* bat CoV HKU9 from the frugivorous Leschenault's rousette (lineage d) (Lau *et al*. 2010b; reviewed by van Boheemen *et al*. 2012 and Woo *et al*. 2012).

Genetic diversity of coronaviruses is multifactorial, involving the infidelity of RNA-dependent RNA-polymerase (RdRp), which has a high frequency of homologous RNA recombination due to unique random template switching during replication, their unusually large genomes, gain and loss of domains, and interspecies jumping events, at least in betacoronaviruses (reviewed by van Boheemen *et al*. 2012 and Woo *et al*. 2012). The poor fidelity of the RdRp, however, is partially offset by the presence of an exonuclease replicase protein, absent in other positive-strand RNA viruses, that appears to serve as a proofreading mechanism (Denison *et al*. 2011). Nevertheless, the mean evolutionary rate due to RdRp in betacoronaviruses is estimated to be 2.37×10^{-4} nucleotide substitutions per site per year. This diversity may promote emergence of viruses with novel traits that adapt to different ecological niches and hosts, sometimes leading to spillover to humans or our domestic animals (reviewed in van Boheemen *et al*. 2012). An example of the former is the finding that HCoV-OC43 is a zoonotic virus of bovine origin that emerged around 1890, most likely from bovine-to-human transmission (reviewed in Woo *et al*. 2012).

5.2 SARS CORONAVIRUS

5.2.1 The history of SARS

The first known cases of SARS occurred in mid-November, 2002, in Guangdong Province, China, and presented as fever and respiratory symptoms, including atypical pneumonia. This was followed about a month later by an independent outbreak originating with a Chinese chef. Several other early clusters in Guangdong or Guangxi Provinces followed a pattern of spread to family members and health care workers and then disappearing after several rounds of human-to-human transmission. Contact with exotic or game animals, often in restaurants or "wet markets," was associated with outbreak initiation. Consumption of exotic animals is generally believed to have health-promoting benefits and is especially common during winter months, a time in which respiratory tract infections are prevalent. A SARS-like-CoV was isolated using nasal or fecal swabs of six masked palm civets (*Paguma larvata*) and one raccoon dog (*Nyctereutes procyonoides*) from a wet market in Shenzhen, China. Such markets bring together many species of animals from different geological locations, caged close to each other in crowded areas where they are exposed to a variety of fecal material. The isolate's full genome is 99.8% identical to the human epidemic strain SARS-CoV Urbani, differing by 18 amino acids in the S protein. Only civets from wet markets were found to be seropositive for SARS-CoV, not those coming from farms or wild-caught

animals (Ge *et al*. 2013). Ferret badgers from these markets in southern China also have a SARS-like CoV (reviewed in Raj *et al*. 2014a). Of note, bats are also commonly found and served in animal markets and restaurants in Guangdong, China (Lau *et al*. 2010a).

In late January 2003, the first "super-spreader" emerged. Such people transmitted disease to large numbers of others, triggering rapid spread of the disease into the community, including those with whom they had only casual contact, such as on public transportation. The disease spread via health care providers and their contacts to Hong Kong, Vietnam, Singapore, and Canada (Hilgenfeld & Peiris 2013). Eventually, over 8000 cases and 774 deaths were reported in 30 countries in five continents during 2002–2003 (Ge *et al*. 2013).

Heroic efforts on the part of health care providers, public health workers, and researchers working together with law enforcement and political bodies brought extremely rapid resolution to the SARS outbreak. By late March 2003, a novel CoV was linked to SARS infection. Within a month, the virus, SARS-CoV, was fully mapped and declared to be the causative agent of this disease. In early July of 2003, the outbreak ended. Two small outbreaks occurred in late 2003–early 2004, linked to either a laboratory or to a live animal market. No further human cases have been reported since then. Epidemiological studies indicate that zoonotic transmission of SARS-CoV has occurred at least twice in China: in Guangdong in November 2002, leading to a large outbreak, and in Guangzhou, in December 2003, in a small outbreak. Sequence analysis of viruses demonstrated that they were not derived from the preceding epidemic (Tan *et al*. 2006).

The process of disease control was aided by a peculiar feature of the infection in which virus numbers in the upper respiratory tract secretions were low early during infection and increased afterwards, becoming most infectious when people were very ill, during hospitalization, thus limiting community exposure. This may be due to the location of the SARS-CoV receptor, which is expressed on pneumocytes deep in the lung, but to a far lesser extent in the upper respiratory tract. The targeting of pneumocytes in the lower respiratory tract may lead to a severe clinical disease course with early onset of respiratory distress, hospitalization, and isolation of patients prior to them producing high virus levels in their respiratory secretions (reviewed in Müller *et al*. 2012). Unfortunately, SARS-CoV is more stable in the environment than most coronaviruses, surviving at lower temperature and lower humidity.

In the areas of the large markets that housed diverse groups of animals, a virus closely related to SARS-CoV was detected in some small mammalian species used as exotic food, such as Himalayan palm civets and raccoon dogs. Workers in those areas had a high prevalence of antibodies to SARS-CoV, even if they did not develop disease, while those workers in other areas of the markets lacked these antibodies. This suggests the existence of a high degree of prolonged exposure of humans to coronaviruses of other mammal species, providing many opportunities for spillover of precursors of SARS-CoV to occur. This is supported by the linkage between SARS acquisition and working in a restaurant that kept and killed these animals.

5.2.2 SARS pathology

The incubation period of SARS is generally 2–10 days, followed by fever, chills, rigor, headache, dizziness, malaise, and myalgia. The respiratory stage of SARS begins with a dry, nonproductive cough with mild nasal discharge. By the time of fever onset, most

patients have abnormal chest radiographs, beginning with subtle peripheral pulmonary infiltrates that progress to bilateral and generalized, with interstitial or confluent infiltrates, with air-space opacities eventually developing. Moderate to severe cases develop dyspnea and hypoxia. In 10–20% of hospitalized patients, mechanical ventilation is required due to progressive immune infiltration of the lungs with diffuse alveolar damage that, nevertheless, fails to clear the viral infection. This eventually culminates in acute respiratory distress syndrome in approximately 16% of SARS patients, associated with a mortality rate of 50%. In addition to damaging the respiratory (including alveoli) and immune systems (including T lymphocytes, monocyte/macrophages, lymph nodes, and spleen), the kidneys, brain, digestive tract, heart, liver, thyroid gland, and urogenital tract are affected (Guo *et al*. 2008). The greatest risk factor for severe disease is being older than 60 years, along with other prognostic factors, including the presence of comorbidities such as diabetes mellitus and cardiac disease, elevations of baseline LDH and ANC, and baseline hypoxemia.

Much of the pathology in SARS may be immune-mediated. Innate interferon (IFN) responses fail to function correctly during inflammatory responses in severe cases and unregulated expression of type I IFNs and the IFN-stimulated chemokines CXCL10 and CCL2 may result in widespread immune dysregulation. Elevated levels of the chemokines IL-8, CCL2, and CXCL10 are found during acute SARS infection and levels of the cytokines IFN-γ, IL-1, IL-6, and IL-12 remain elevated for at least 2 weeks. Increased amounts of CXCL10, CXCL9, and IL-8 early during the disease are associated with adverse outcome (reviewed by Cameron *et al*. 2008; Thiel & Weber 2008). Severe SARS patients also had higher levels of CXCL10 and CCL2 during the late phase of the disease, together with lower levels of IL-12p70 and TNF-α than was seen in patients with less severe illness (Cameron *et al*. 2008). The immune response to SARS-CoV infection is discussed in greater detail in Chapter 1.

5.2.3 Viral and cellular proteins and their role in entry into the host cells

As stated in Section 5.1, coronaviruses have the one of the largest reported positive single-stranded RNA genomes. The SARS-CoV genome is 27.8 kb and contains fourteen open reading frames (ORFs) that code for at least 28 proteins (reviewed in Hilgenfeld & Peiris 2013). Their spike (S) protein is a type I transmembrane protein that protrudes from the viral surface, giving it a crown-like ("corona") appearance. The S protein contains a distinctive N terminus (S1) in additional to a conserved C terminus (S2). S1 contains the receptor binding domain (RBD) that determines the virus's host specificity. S2 is responsible for viral fusion. Both S1 and S2 are produced as a single polyprotein that must be cleaved by host proteases before the coronaviruses can enter host cells. The ability of the S protein to be cleaved by a particular host's enzymes helps to determine viral host selection (reviewed by Y. Yang *et al*. 2014). SARS- and MERS-CoV use the human type 2 transmembrane serine protease (TMPRSS2). The host endosomal protease cathepsin L is also necessary for S protein cleavage. The angiotensin-converting enzyme-2 (ACE2) is the host cell receptor that binds to the RBD portion of human SARS-CoV. HCoV-NL63, an aminopeptidase N (APN), acts as the cellular receptor for HCoV-229E CoV. DPP4, a conserved ectopeptidase that cleaves dipeptides from hormones, chemokines, and cytokines, is the MERS-CoV receptor.

DPP4's enzymatic activity is not critical for cellular infection by MERS-CoV since inhibition of its enzymatic activity does not block infection (reviewed in Wang *et al*. 2013). Other CoV structural proteins include the nucleocapsid and matrix proteins and the envelope glycoprotein.

SARS-CoV is well-adapted to the human ACE2 receptor and is unable to infect bat cells (reviewed in Müller *et al*. 2012). Of note, human SARS-CoV and the closely related civet SARS-CoV S protein cannot use the Pearson's horseshoe bat (*Rhinolophus pearsoni*) ACE2 protein as a receptor. The crystal structure of the human SARS-CoV RBD complexed with human ACE2 suggests that this restriction is due to truncations in the RBD of bat SARS-like-CoV S protein (reviewed by Hou *et al*. 2010). By contrast, the ACE2 of the bats *Myotis daubentoni* and *Rhinolophus sinicus* do support SARS-CoV entry, suggesting that these bats might be susceptible to human SARS-CoV infection. It should be noted, however, that viral entry utilizing the bat ACE2 receptor differs in efficiency with that of human ACE2 protein due to the mutation of several key amino acids. Genetic diversity of bat ACE2 is also greater than that displayed by other known human SARS-CoV-susceptible mammals, suggesting that other bat species may or may not act as reservoirs for viruses similar to SARS-CoV (Hou *et al*. 2010). In addition to the inability of SARS-CoV to bind the ACE2 protein of most bats, bat SARS-like CoV S proteins expressed by an HIV-based pseudovirus are also not able to support infection of cell lines expressing human, civit, or the bat *R. pearsonii* ACE2, but replacement of amino acids 310–518 converts the SARS-like-CoV S to a form in which it is able to bind human ACE2 (Ren *et al*. 2008). Unfortunately, appropriate cell lines from *Rhinolophus* were not available for testing at the time of the study.

Bat ACE2 are identical in size to the human ACE2 (805 amino acids) and have an amino acid identity of 80–82% to human and civet ACE2. The amino acid identity of ACE2 varies among different bat families, ranging from 78 to 84% identity, and within the genus *Rhinolophus*, from 89 to 98%. The major sequence variation among bat ACE2s is within the N-terminal region, which contains the SARS-CoV-binding region (Hou *et al*. 2010). ACE2 from *M. daubentonii* and *R. sinicus* from the Hubei province of China (Rs-HB) permitted cellular infection by a pseudovirus bearing the human SARS-CoV S protein, but not the ACE2 protein of *R. sinicus* from the Chinese Guangxi province or the ACE2 of *R. ferrumequinum, Rhinolophus macrotis, R. pearsoni, Rhinolophus pusillus*, or *Hipposideros pratti* bats. Additionally, ACE2 of *R. sinicus* from the Hubei province contains structural features that make it a low affinity receptor for human SARS-CoV.

SARS-CoV has eight accessory proteins whose length varies greatly (39–274 amino acids). Accessory gene functions are not essential for replication in cell culture and thus most of them may not be under as great a level of selective pressure as other genes. In animal models, however, they help to determine virulence, block cell cycle progression, induce apoptosis, and block innate immune system signaling *in vivo* (Tan *et al*. 2006; reviewed by van Boheemen *et al*. 2012). Because of a low degree of selective pressure, several accessory genes undergo rapid evolution that may be critical for virulence. ORF8 of CoV from palm civits and from humans early during the SARS outbreak only encoded one protein, but by early 2003, the genome of human SARS-CoV lost 29 nucleotides and subsequently encoded two separate accessory proteins, 8a and 8b. This event may be at least in part responsible for the increased efficiency of human-to-human

transmission that initiated the epidemic stage of the SARS outbreak (Tan *et al*. 2006; reviewed by Hilgenfeld & Peiris 2013).

Another accessory protein, 3a, is an integral membrane protein expressed on the viral surface. Its external domain elicits strong antibody responses that allow removal of infected cells by the complement component of the host's innate immune response. The 3a protein is of particular interest since it interacts intracellularly with the S protein and may play a role in modulating S protein surface expression. The genes for both S and 3a proteins appear to be under positive selection during virus evolution (reviewed by Tan *et al*. 2006). Viral 3a may influence the up-regulation of fibrinogen seen in immune cells of infected individuals (reviewed in Tan *et al*. 2006). Excessive production of fibrinogen may increase cytokine production by the host's adaptive immune response and alter the pro-coagulant and fibrinolytic balance. This may result in the dysregulated coagulation and fibrin polymerization pathways seen in the lung pathogenesis of most SARS patients.

Viral ORF1 is approximately two-thirds of the SARS-CoV genome and encodes two huge polyproteins, pp1a (approximately 486 kDa) and pp1ab (approximately 790 kDa), which are cleaved into 15–16 nonstructural proteins by two cysteine proteases, a papain-like protease (PLpro) and the main protease (M_{pro} or 3CLpro). M_{pro} is the target of several anti-coronavirus drug candidates. The majority of the viral nonstructural proteins in conjunction with some host components assemble the viral replication and transcription complex in double-membrane vesicles as well as other unusual membrane structures derived from the endoplasmic reticulum membrane. Afterwards, a nested set of subgenomic mRNAs is produced and translated into the structural and accessory proteins which, together with newly synthesized genomic RNA, are assembled into progeny virions. These then bud through the membranes of the intermediate endoplasmic reticulum-to-Golgi compartment and leave the host cell by exocytosis (reviewed by Hilgenfeld & Peiris 2013). One of the conserved nonstructural proteins, the RNA-dependent RNA polymerase RdRp, RdRp, has been the target of much of the comparative sequencing efforts used to develop hypotheses concerning the relatedness of SARS- and MERS-CoV to a variety of coronaviruses from bats and other animals.

MERS-CoV generates less of a proinflammatory response in differentiated bronchial epithelial cells *in vitro* than SARS-CoV does, perhaps partially explaining why it replicates to a lesser extent in these tissues than SARS-CoV. MERS-CoV also targets type I and type II alveolar cells of the lungs. This may be significant in the disease pathology since type II cells are important for tissue repair. HCoV-229E, a milder human pathogen, does not replicate in lung tissue, while the highly pathogenic influenza A (H5N1) virus, associated with pneumonia, does (reviewed in Mackay & Arden 2015).

5.2.4 SARS in civits and raccoon dogs

RNA of coronaviruses that are very closely related to SARS-CoV was isolated from Himalayan palm civets, a raccoon dog, and humans in a live-animal market in Guangdong, China. When comparing healthy wild-animal traders, people involved in animal slaughter, and vegetable traders, seropositivity for SARS-CoV was 40, 20, and 5%, respectively. Full-genome sequencing of human and palm civit SARS-CoV isolates showed a 99.8% homology. Three isolates from palm civits (originally from different geological locations) were phylogenetically distinct, having up to 18 nucleotide differences.

Five human SARS-CoV isolates from separate geographical sites differed by 14 nucleotides. The S genes of three civits' and 1 raccoon dog's viruses had eight nucleotide differences and there were 20 differences among 11 human SARS-CoV isolates from Hong Kong, Guangdong, Canada, and Vietnam. Interestingly, while 70% of the polymorphisms among the human viruses were nonsynonymous mutations, only 25% were so in the animal viruses. Eleven consistent nucleotide signatures appear to have differentiated the animal and human viral isolates. All but one human isolate tested in this study lacked a 29-nucleotide sequence in ORF8 that was present in all animal isolates (Guan *et al*. 2003). The ORF8 of human strains from later stages of the epidemic increased viral replication and induced apoptosis via a mitochondria-dependent pathway, while that from civet and early human isolates was instead found in the endoplasmic reticulum (reviewed in Lau *et al*. 2010a).

Interestingly, a 2007 study found that pseudoviruses expressing four different civet-CoV S genes containing distinct RBDs infected cells expressing human ACEs and infected human cells with 90–95% less efficiency than those expressing S genes from human SARS-CoV. This has been suggested to be because these civet coronaviruses contain either one or the other of the critical RBD residues 479 N and 487 T, but not both (Liu *et al*. 2007). Since 479 N was found in eight civet coronaviruses, the additional mutation 487 T may be important for adapting to entry into human cells. Three human SARS-CoV isolates lack 487 T and only caused mild human infections with low transmissibility, suggesting an independent cross-species event (Liu *et al*. 2007).

Sheahan *et al*. (2008), however, reported that the SARS human epidemic Urbani viral isolate grew similarly in cells expressing either human or civit ACE2, while a recombinant human SARS-CoV virus expressing the S protein from the civit-CoV SZ16 isolate only grew in cells expressing the civit ACE2. Civit and human ACE2 differ by only two amino acids. Recombinant SZ16-S mutant viruses K479N and D22, bearing mutations at three specific sites, however, grew well in cells expressing human ACE2 but not civit ACE2. This suggests that the evolutionary pathway that promoted efficient human ACE2 binding simultaneously abolished efficient civit ACE2 interaction. Since the human epidemic Urbani SARS-CoV strain had dual species tropism, the virus may have evolved high affinity for civit and human ACE2 receptors by repeated passages between human and civet hosts (Sheahan *et al*. 2008). This report also supports the contention that the civit-CoV SZ16 strain is closely related to at least some human SARS-CoV isolates. Interestingly, civets infected with human-tropic SARS-CoV develop disease that is similar to that seen in infected humans (Sheahan *et al*. 2008). Taken together, these findings suggest that human CoV infection likely originated from coronaviruses of palm civits.

5.2.5 Relatedness of bat SARS-like CoV to SARS-CoV

Great diversity of SARS-like coronaviruses is present in *R. sincus*. Yuan *et al*. (2010) isolated a strain from *R. sinicus* that contains the distinctive 579-nucleotide deletion in the nsp3 region that is a characteristic of human SARS-CoV from the late-phase epidemic, but is not present in most bat isolates. Phylogenetic analysis of ORF1 suggests that the SARS-like CoV of *R. sincus* is more closely related to SARS-CoV than isolates from other *Rhinolophus* species. Importantly, *R. sincus* is an extremely common species of this genus in China. The SARS-like CoV sequences from *R. sincus* contain two

topologically distinct clusters: Rp3, HKU3, and Rs806 in clade 1 and Rs672 in clade 2 throughout southern China. Orf1a and Orf1b of Rs672 are more similar to that of the human SARS-CoV than to that of other bat SARS-like coronaviruses, however, a different region is more similar to bat SARS-like CoV than to that of human SARS-CoV, suggesting a possible recombination between bat and human SARS-CoV, as had been previously reported for the Rp3 isolate. Two different analyses suggest that the potential recombinatorial breakpoint is immediately after the start codon of the spike gene at the same position as that found in Rs806. The genome regions upstream and downstream of this point are designated the major and minor parental regions. The major parental region of RS672 is phylogenetically closer to human SARS-CoV than to bat viruses and the minor parental region of Rs672 clusters with the bat SARS-like CoV lineage. Both Rs672 and Rp3 may have evolved from a common ancestor, however, Rs672 and Rp3 and their hosts may have diverged a relatively long time ago. The potential direct or indirect interspecies transmission between bats and the onset of the SARS epidemic is estimated to be 4.29 years (Yuan *et al*. 2010).

Between 2004 and 2008, 9.4 and 6.3% of the insectivorous *R. sincus* bats from Hong Kong and Guangdong, China, respectively, contained SARS-like CoV in their digestive samples. These bats can migrate from 1.86 to 17 km. The positive bats appear to be healthy, but have lower body weights than bats without signs of infection. Viruses are cleared by the bat immune system within 2 weeks to 4 months. Frequent recombination occurs between Rp3 from Guangxi, China, and Rf1 from Hubei, China, with the breakpoint at the ORF1/S junction. Molecular clock analysis indicated that the bat strains diverged in 1972, followed by the divergence of civet and bat strains in 1995. This supports the hypothesis that *Rhinolophus* bats act as reservoirs for recombination between SARS-like CoV strains from different geographical locations that are within reachable foraging range and that civet SARS-like CoV, such as strain SZ3, may have arose by recombination similar to that occurring between bat Rp3 and Rf1 (Lau *et al*. 2010a).

At least five *Rhinolophus* species in mainland China and Hong Kong host SARS-like coronaviruses (betacoronaviruses of lineage b): *R. sincus*, *R. pearsonii, R. ferrumequinum, R. macrotis*, and *R. pusillus*. These SARS-like CoV isolates are HKU3-1, HKU3-2, Rp3, Rf1, and Rm1 (reviewed by Ren *et al*. 2006). Bat beta-CoV Rf1 and Rm1 isolates were sequenced from *R. ferrumequinum* and *R. macrotis* bats and have an overall genome sequence identity of 88–92% between themselves and human/civet isolates. The greatest variation exists in the genes encoding ORF1, ORF3a, S, and ORF8 (Ren *et al*. 2006). Bat CoV Rf1 may be an evolutionary intermediate between bat lineage b betacoronaviruses and those from humans and civets. The latter two coronaviruses have an ORF3b of 154 amino acids that is absent from most bat SARS-like CoV, while in the corresponding region of the Rf1 genome, there were two ORFs of 113 and 32 amino acids (Ren *et al*. 2006). The sequence identity of the S genes of bat and human or civit isolates is 76–78%, while that of the S1 domain is 63–64%. Bat isolates additionally have a 6 amino acid insertion and three deletions of various lengths in the S1. Two of the deletion sites are in the RBD and overlap with the RBM (Ren *et al*. 2006), calling into question the ability of these bat betacoronaviruses to serve as the predecessors of SARS-CoV since these regions are vital for the binding of host cells.

Upon the discovery of a beta-CoV, lineage b, in *Hipposideros larvatus* bats from Southeast Asia, it has been hypothesized that the presence of beta-CoV in *Rhinolophus*

bats was the result of a spillover from those infecting *Hipposideros*, its sister taxon (Gouilh *et al*. 2011). Unlike the short time periods of persistence in *Rhinolophus* bats, the novel bat beta-CoV persisted for 18 months in a *Hipposideros* bat colony. The latter colonies might be more tolerant of betacoronaviruses over long periods of time or the betacoronaviruses of *Rhinolophus* bats may have acquired factors that limit their virulence. Studies of bat ancestors of civit and human SARS-CoV should perhaps be expanded to the *Hipposideros* genera as well, especially since studies have not focused as heavily upon this bat group (Gouihl *et al*. 2011). It should be noted that beta-CoV infection may be confined to only a few *Hipposideros* species since *Hipposideros armiger* dwelling in a separate site in the same cave were not infected. Alternatively, more direct contact between the bat groups may be required for interspecies transmission.

Molecular clock analysis suggests that bat and civet/human strains diverged 4–17 years before the large human outbreak (reviewed in Lau *et al*. 2010a). SARS-related coronaviruses appear to have been transmitted from civets to humans, with horseshoe bats being perhaps the primary host. Civet SARS-related CoV may have also arisen from recombination of different strains of SARS-like bat CoV from different locations in China (reviewed in Woo *et al*. 2012). Analysis of nonsynonymous and synonymous substitution rates (K_a and K_s, respectively) suggest that SARS-like coronaviruses in bats are not under positive-selection pressure and have evolved independently for a relatively long time. Human and civet isolates appear to have undergone a strong positive selection, suggesting recent interspecies transition (Ren *et al*. 2006).

Whole-genome sequencing detected two novel bat coronaviruses (RsSHC014 and Rs3367) from the Rhinolophidae family of horseshoe bats in Yunnan, China (Ge 2013). Their genes have a high degree of homology in the RBD of the S protein from SARS-CoV. RsSHC01014 has 99.9% nucleotide homology with the WiVi isolate from bat feces, which utilizes the ACE2 of horseshoe bats, civits, and humans during entry into its target cells. Rs3367 is also able to use human ACE2 for cell entry (Ge *et al*. 2013). A novel SARS-like beta-CoV (LYRa11) was found in *Rhinolophus affinis* in Yunnan, China, which has spherical, enveloped virus-like particles with surface spikes, but nevertheless does not have the typical petal-shaped CoV morphology. Infectious viruses were not able to be isolated from rectal samples and only a few CoV-like particles with the unusual spike morphology were found (He *et al*. 2014). LYRa11 has a 98.4% nucleotide identity with the conserved *RdRp* gene of bat coronavirus Rs3367. Full genome sequencing of LYRa11 indicates 91% nucleotide identity with SARS-CoV, with the variable *S* gene having 99% identity. The genome contains 29 805 nucleotides (slightly larger than SARS-CoV) with 40.7% G+C content and 13 ORFs. It has 83.3–84.0% amino acid identity with S1 of human and civet SARS and bat Rs3367 and a low degree of identity with other bat SARS-like CoV. The RBD has 92.5–94.6% amino acid identity to human and civet SARS-CoV and 95.1% identity to Rs3367, while other bat SARS-like CoV has only 58.7–61.3% amino acid identity to human and civit SARS-CoV. LYRa11, however, lacks ORF4 of the human SARS-CoV isolate Tor2 and bat CoV Rs3367 (He *et al*. 2014).

Within the RBD (319–518 amino acids) lies the receptor binding motif (RBM) (426–518 amino acids), the most variable region and that which determines host selection. Another bat isolate, BM48, from the Bulgarian *Rhinolophus blasii* bat has a four amino acid deletion in this critical RBM region, as well as a greater amino acid difference

to human and civet viruses in comparison with LYRa11. Other bat SARS-like coronaviruses have a 17–18 amino acid deletion in the RBM. By contrast, LYRa11 and Rs3367 have no deletion in the RBM (He *et al*. 2014). The RBM contains two critical amino acids involved in receptor recognition and binding enhancement. Substitution of both of these amino acids (but not either one alone) stops binding to the ACE2 receptor. Since both bat Rs3337 and LYRa11 have mutations at only one of those critical amino acids, both isolates are still able to bind to human ACE2. The two viral isolates are distinct since Rs3367 contains ORF 4 and was isolated over 350 km from the location of LYRa11(Kumming). Of interest, coinfection of host cells with two distinct coronaviruses may lead to genomic recombination. This may have been involved in the origins of RS3367, LYRa11, and human SARS-CoV – the "Gap-Filling virus" hypothesis (Kumming).

In order to further explore the relatedness of the bat and human beta-CoV, their mechanisms of avoiding the host innate immune response was compared, with particular interest in IFN, since this host cytokine is among the most powerful means of controlling or eliminating viral infections. Human SARS-CoV contains at least five proteins (products of ORF3b, ORF6, the nucleocapsid protein and several products of ORF1) that act as antagonists of either IFN production or signaling pathways. Homologs of SARS-CoV ORF3b in the bat SARS-like coronaviruses Rf1, Rm1 and Rpl contain different C-terminal truncations (Zhou *et al*. 2012). The three bat-derived ORF3b proteins vary in their ability to suppress IFN. ORF3b of bat CoV Rf1 is toxic to human cells without inducing apoptosis, while that of bat CoV Rp1 does not antagonize IFN, and that of bat CoV Rm1 is a potent IFN antagonist in human cells that acts by blocking IRF3 nuclear translocation and preventing activation of the IFN-β gene promoter. This is the same mechanism of action used by the ORF3b protein of human SARS-CoV (Zhou *et al*. 2012). The nucleocapsid protein of bat CoV Rm1 is also a functional IFN antagonist.

5.3 MERS CORONAVIRUS

5.3.1 MERS pathology

MERS emerged in 2012 in Saudi Arabia. The mean incubation period is approximately 5 days, and 95% of cases become symptomatic within 13 days, although subclinical or asymptomatic infection may occur and one health care worker shed virus for 42 days in the absence of overt illness (reviewed by Mackay & Arden 2015). The most common symptoms are fever, fever with chills or rigors, cough, shortness of breath, and myalgia. MERS can, however, cause severe lower respiratory tract infection and renal failure and has a much higher fatality rate than SARS (approximately 30%). Gastrointestinal symptoms, such as diarrhea, vomiting, and abdominal pain, may also occur. In experimentally infected dromedary camels, lesions are present in the epithelium of both upper and lower respiratory tracts, with viable virus recoverable from both locations (reviewed by Khalafalla *et al*. 2015). MERS-CoV replicates efficiently in human respiratory tissues and also targets alveolar epithelial cells and the endothelium of lung blood vessels. In the lungs of experimentally infected macaques, MERS-CoV was found primarily in type I and type II pneumocytes (reviewed in Hilgenfeld & Peiris 2013; van Doremalen *et al*. 2014b).

Approximately 75% of human patients have one or more underlying medical condition, such as diabetes; chronic kidney, heart, or lung disease; hypertension; asthma; obesity; smoking; steroid use; malignancy; recent surgery; or co-infection with influenza A virus, parainfluenza virus, herpes simplex virus, or pneumococcus (Abdel-Moneim 2014). Outbreak index cases have been traced to Saudi Arabia, Jordan, Qatar, and the United Arab Emirates, and travel-associated cases have been found in an ever-expanding number of locations, including France, Germany, Italy, Tunisia, the UK, the US, South Korea, and Thailand. Fatal cases of MERS tend to occur in those having underlying illnesses, especially those who are immunocompromised. Secondary transmission has become a major means of transmission to healthy family members and in hospitals to health care providers, to other patients, and even to those paying brief visits to a ward with an undiagnosed MERS patient. The ability to undergo human-to-human transmission appears to be increasing over time and was the sole factor operating in the large outbreak in South Korea.

5.3.2 Viral and cellular proteins and their role in entry into the host cells

The mammalian host cell receptor for the MERS-CoV S protein is dipeptidyl peptidase IV (DPP4 or CD26), a type II transmembrane protein expressed in the human respiratory tract, kidneys, small intestine, liver, parotid gland, spleen, testes, prostate, and activated immune cells. It is conserved among many animal species, including nonhuman primates, dromedaries, sheep, cows, and bats (reviewed in Hilgenfeld & Peiris 2013). The MERS-CoV S protein's S1 core domain is responsible for DPP4 recognition and high affinity binding to host cells. The S2 domain serves as a C-terminal 240-amino acid RBD composed of amino acids 367–606. The external subdomain portion of viral S2 binds to the host DPP4 receptor and has thus been designated the RBM (Lu *et al*. 2013; Wang *et al*. 2013). Several of the amino acids involved in binding the MERS-CoV S protein are also crucial in binding to the human enzyme adenosine deaminase (ADA), a natural DPP4 ligand. Recombinant forms of ADA are able to compete with the MERS-CoV S1 region for DPP4 binding to cell lines *in vitro* and inhibit their infection. ADA's normal functions include differentiation and maturation of lymphoid cells of the adaptive immune system by stimulating dendritic cells, costimulating T helper lymphocytes, and increasing production of proinflammatory cytokines that may be involved in MERS pathogenesis (reviewed in Raj *et al*. 2014a). The ability of recombinant ADA to limit *in vitro* infection of cells may aid in the development of other antagonists for DPP4-mediated entry of MERS-CoV, thus limiting disease severity. Five human MERS-CoV accessory proteins share homology only with those from bat HKU4 and HKU5 coronaviruses (Raj *et al*. 2014b). As with SARS-CoV, MERS-CoV has mechanisms to avoid triggering the host's interferon response, but unlike SARS-CoV, it remains sensitive to any interferon that is produced (reviewed in Hilgenfeld & Peiris 2013).

MERS-CoV has been subdivided into several clades. Clade A is only known to contain variants derived from African green monkey kidney Vero cells, cell-culture passaged EMC/2012 variants, two Jordan-N3 variants, but no camel-derived MERS-CoV variants. Clade B contains Bisha 1, directly sequenced from the upper respiratory tract of a human primary MERS case, having a 115 nucleotide difference from the EMC/2012 variants produced after culturing MERS-CoV from this patient *in vitro*

(reviewed in Mackay 2015). Clade C contains a very divergent variant derived from an Egyptian dromedary, NRCE-HKU205|Nile|2013, most likely imported from Sudan. An additional virus from a *Neoromicia capensis* bat, NeoCoV, is more closely related to MERS-CoV than previous bat sequences and may link camel and bat viruses as members of the same CoV species (described in more detail below; reviewed in Mackay & Arden 2015). Nine or more of the human MERS-CoV genomes contain amino acid substitutions in the RBD and several of the substitutions appear to be markers of adaptive change. An *in vitro* analysis did not, however, demonstrate differences in viral shedding, replication, or immune escape among the tested MERS-CoV variants (reviewed in Mackay & Arden 2015).

5.3.3 MERS-CoV and spillover from domestic livestock

MERS-CoV transmission to humans as a zoonotic spillover has been convincingly traced to exposure to live dromedaries or their raw milk or urine. In addition to the presence of high neutralizing antibody titers to MERS-CoV in many dromedaries throughout the Middle East, viral genomes identical to that of human MERS-CoV have been isolated from these animals. In one instance, a human isolate was identical to that obtained by a nasal swab from a sick dromedary for which the patient had cared (Haagmans *et al*. 2014).

Cows, goats, sheep, and dromedary camels are the primary sources of meat and milk in Jordan, Saudi Arabia, and United Arab Emirates. Two species of camels exist: one-hump dromedaries (*C. dromedarius*) and two-hump Bactrian camels (*C. bactrianus*). Dromedaries are found in hot desert regions of the Arabian Peninsula, Middle East, Afghanistan, central Asia, India, and parts of Africa. Dromedary density is highest in and around the Greater Horn of Africa (Ethiopia, Somalia, Kenya, Sudan, and South Sudan) and these camels are exported to other regions. In the Arabian Peninsula, Yemen has the highest dromedary density, particularly the Ha'il region, however known cases of human MERS are less common in this region than in Saudi Arabia. Human-dromedary contact occurs at festivals, races, sales, and parades (Mackay & Arden 2015). MERS-CoV infection in dromedaries is asymptomatic or results in only mild respiratory symptoms, so its presence may be undetected (reviewed in Gossner *et al*. 2014). Bactrian camels inhabit the colder steppes of Mongolia, Central Asia, Pakistan, and Iran.

Experimental infection of dromedaries with MERS-CoV leads to a mild (nasal discharge and slight fever), transient, primarily upper respiratory tract infection (Adney *et al*. 2014). The camels shed large amounts of infectious virus and RNA in their nasal secretions until 7 days after infection and viral RNAs were detectable for up to four additional weeks. Despite the detection of small levels of MERS-CoV RNA by PCR in exhaled breath, no infectious virus was found at that time (reviewed in Khalafalla *et al*. 2015).

Very little virus is present in oral samples and may result from nasal drainage. No RNA was detected in fecal, urine, serum, or blood samples. Infectious virus was detected in several tissues from a camel euthanized on day 5 post-infection, but not from camels euthanized at days 28 or 42. No infectious virus was present in the digestive tract (abomasum, forestomachs, duodenum, jejunum, colon, or rectum), liver, spleen, kidney, bladder, or heart of these animals. Infectious virus was confined to tissues of the upper respiratory tract (primarily the nasal turbinates, but also the olfactory epithelium,

pharynx, and larynx), lower respiratory tract (trachea and in one of the lung lobes), and lymph nodes (retropharyngeal, mediastinal, mesenteric, and tracheobronchial). Mild to moderate inflammatory lesions, comparable with that caused by the common cold among humans, were present in the pseudostratified columnar epithelial cells lining the upper and lower respiratory tract, but not in the alveoli. The location of MERS-CoV in the upper respiratory tract may at least partially explain the lack of systemic illness in naturally infected camels as well as the means of camel-to-camel and camel-to-human transmission (Adney *et al*. 2014).

In a large study of sera from these domestic livestock, all sera from camels from Oman ($n=50$) contained neutralizing antibodies against the S1 region of the MERS-CoV spike protein, while only 14% from the Canary Islands contained these antibodies ($n=105$). Dutch or Spanish sheep, goats, cattle, and other camelids (2 Dutch Bactrian camels, 2 llamas, 6 alpacas, and as well as 2 Bactrian camels, 5 llamas, 18 alpacas, and 2 guanacos from Chile) were seronegative (Chan *et al*. 2015). Antibody titers ranged from 1/320 to 1/2560 for Omani camels, but were only 1/20 to 1/320 for those from the Canary Islands (Reusken *et al*. 2014). Unfortunately, this study did not examine sera from sheep, goats, or cattle from MERS-endemic regions. Studies published in 2013 and 2014 failed to detect MERS-CoV-specific antibodies in sheep, goats or cattle in Jordan or Saudi Arabia (reviewed in Gossner *et al*. 2014).

Antibodies to MERS-CoV in dromedaries have also been detected in Jordan, Egypt, United Arab Emirates, Saudi Arabia, Qatar, Nigeria, Tunisia, Ethiopia, Kenya, Sudan, South Sudan, and the Canary Islands (Perera *et al*. 2013; Reusken *et al*. 2013; Corman *et al*. 2014; Gossner *et al*. 2014; Reusken *et al*. 2014). The virus appears to have been circulating in dromedaries by 1992 in Saudi Arabia and 2003 in the United Arab Emirates (reviewed in Gossner *et al*. 2014). Many of these animals were also seropositive for the bovine coronavirus, known to widely circulate among camel populations, but they lacked antibodies against SARS-CoV. Some of these samples were collected in 2009 or as early as 2003, indicating that the virus was wide-spread in dromedary populations before the MERS-CoV outbreak in humans (Reusken *et al*. 2014). In a separate study, 80% or more of dromedaries in Somalia and Sudan were seropositive for MERS-CoV in 1983 and similar results were found in Egypt in 1997 (Müller *et al*. 2014). Due to the high levels of civil unrest and war in the former countries, it is possible that human MERS cases have been present in the region and undetected for several decades (Müller *et al*. 2014).

RNA from two to three MERS-CoV genes was detected in nasal swabs from 6 of 14 dromedaries from a farm in Oman. There was 100% identity between a tested S protein fragment from three camels and S protein from several human MERS-CoV isolates, including that of a patient related to that farm, but some sequence differences were found in ORF1 and a MERS-CoV EMC isolate. No viral RNA was found in rectal swabs and fecal samples. All animals had antibodies to MERS-CoV antigen, but not to SARS-CoV or human coronavirus HCoV-OC43 (Haagmans *et al*. 2014).

The owner of a small herd in Saudi Arabia developed a fatal case of MERS after contact with mucus secretions from an ill dromedary. Three of his other eight animals were also ill. Viruses isolated from patient and camel nasal swabs were grown in culture. Full genome sequencing of the cultured patient and human MERS-CoV RNA were identical. No MERS-CoV RNA was recovered from the camel's nasal swabs 28 days later, suggesting a transient, acute infection since all of the ill camels were healthy

several weeks later. RNA was not recovered from milk, urine, or rectal samples from any of the camels in this study (Azhar *et al*. 2014), however, there have been several reports of MERS-CoV in camel feces in Saudi Arabia and in feces and milk in Qatar (reviewed by Gossner *et al*. 2014).

Dromedary infection in Saudi Arabia in 2013–2014 varied by season, with RNA present during the cooler months (November–January) and decreasing with warming weather, reaching a low point in May (Khalafalla *et al*. 2015). Cooler temperatures enhance survival of coronaviruses outside of the host. The cool season is the time of greatest circulation of human respiratory viruses as well as corresponding to the peak of dromedary calving season in Saudi Arabia (Khalafalla *et al*. 2015). Gossner *et al*. (2014), however, found a different seasonal pattern in human case incidence: the first primary case was detected in April 2012, an increase in new human cases occurred around April and May 2013, and a third increase in April 2014. Interestingly, calves are first weaned in March–April at the beginning of the hot season. The calves are very susceptible to diarrhea at this time and infected calves can excrete MERS-CoV in their feces. Milking is usually performed manually and, if teats are not properly cleaned, infected feces from calves may enter into milk consumed by humans (reviewed by Gossner *et al*. 2014).

A large study of more than 750 dromedaries in Dubai demonstrated that more than 96% of adult dromedaries (over 4 years old) were seropositive, as were 85% of calves (less than 1 year old). MERS-CoV RNA was detected in only in nasal swab specimens from dromedaries less than 4 years of age, primarily in calves. Viral isolation from animals in Dubai and Saudi Arabia showed similar age discrimination, suggesting that calves are much more likely to become transiently infected than older animals (Khalafalla *et al*. 2015; Wernery *et al*. 2015). Slaughtering of camels usually involves adults (over 5 yearsold), perhaps accounting for the relative lack of MERS risk for slaughter-house workers (MacKay & Arden 2015). MERS-CoV RNA was detected in 29% of nasal swab samples from live dromedaries and 62% of lung tissue samples from carcasses of healthy animals (Khalafalla *et al*. 2015). MERS-CoV detection is enhanced in human lower respiratory tract samples and is found there for approximately 1 month. During that time, oronasal swab samples tested negative (reviewed in Khalafalla *et al*. 2015). Testing only nasal swabs may therefore fail to detect infected persons or animals.

Cell lines from goats and camels are able to support infection and efficient replication of MERS-CoV (Eckerle *et al*. 2014). A 2013 search of a number of different animal species in Oman, Egypt, and the Canary Islands found MERS-CoV neutralizing antibodies in dromedary camels (Perera *et al*. 2013; Reusken *et al*. 2013). Human kidney cancer, human alveolar adenocarcinoma, bat and goat kidney and lung, and dromedary umbilical cord supported MERS-CoV replication. Viral nucleoprotein was also produced by many experimentally infected mammalian cells, including human *ex vivo* bronchial and lung tissue and embryonic lung fibroblasts, gastrointestinal, liver, and histiocytoma cells (reviewed by Mackay & Arden 2015).

5.3.4 Relatedness of bat-CoV to MERS-CoV

In June 2012, a lineage c beta-CoV, HCoV-EMC/2012 (with variants known as England-Qatar, Jordan-N3 and England 1 and, currently, as MERS-CoV), was isolated from a patient from Saudi Arabia with a fatal case of acute respiratory distress syndrome and

multiple organ dysfunction syndrome with renal failure (Ge *et al*. 2013). A second human case observed 3 months later involved a hospitalized patient from Qatar. The MERS-CoV genome contains between 30 106 and 30 119 nucleotides. It has at least ten predicted ORFs, nine of which appear to be expressed from a nested set of seven subgenomic mRNAs. It has a G+C content of 41% (Woo *et al*. 2012).

At the time, MERS-CoV appeared to be most closely related to the bat coronaviruses HKU4 and HKU5, isolated from *T. pachypus* and *P. abramus*, respectively, in Hong Kong. The latter bat species is widely distributed, not only in China, but also Russia, the Korean peninsula, Japan, Vietnam, Burma, India, and Saudi Arabia and neighboring countries in the Middle East (reviewed in Lau *et al*. 2010b). HKU4 has 30 286–30 316 nucleotides and HKU5 has 30 482–30 488: their G+C contents are 38 and 43%, respectively (Woo *et al*. 2012). MERS-CoV has only 66.3% nucleotide and 66.1% amino acid identity and 63.8% nucleotide and 63.5% amino acid identity with the S proteins of HKU4 and HKU5, respectively (van B 2012). The major difference between human MERS-CoV and bat HKU4 and HKU5 lies in the region between the *S* and *E* genes: MERS-CoV has five ORFs, rather than four found in the bat coronaviruses (Woo 2012). The *RtRp* gene is generally much highly more conserved among coronaviruses and human MERS-CoV has amino acid identities of 89% and 92% with bat HKU4 and HKU5, respectively (van Boheemen *et al*. 2012). Molecular clock analysis indicates that HKU4 and HKU5 diverged from a common ancestor with MERS-CoV hundreds of years ago. Furthermore, complete sequencing of *RdRp, S*, and nucleocapsid genes of 13 HKU4 and 15 HKU5 strains showed that these viruses are stably evolving in each of their bat host species (Lau *et al*. 2010b). Another beta-CoV, VM314, was isolated in 2008 from a *Pipistrellus pipistrellus* bat in the Netherlands. This bat virus has an 88% identity with MERS-CoV in a *RdRp* 332-nucleotide fragment (reviewed in van Boheemen *et al*. 2012). It should be noted that this bat species is also found in Saudi Arabia.

Considerable amino acid variance also exists between bat HKU4 and HKU5 coronaviruses and human MERS-CoV in the RBD region, crucial to host cell binding and tropism (54.4 and 52.9% identity, respectively) (Lau *et al*. 2013). HKU5 additionally has two deletions in the RBM, an especially critical region of the RBD, thus making it even less likely be a progenitor for MERS-CoV (Wang *et al*. 2013). Even the more closely related HKU4 has only 40.8% amino acid identity in the RBM and contains an insertion not present in MERS-CoV. Nevertheless, HKU4's RBD, but not that of HKU5, is able to bind the human DPP4 cellular receptor. The K_D of binding is 35.7 mM, however, about three orders of magnitude lower binding affinity than that of the MERS-CoV RBD. HKU4 binds slightly better to a bat DPP4 than does MERS-CoV, but it should be noted that the bat DPP4 used in the study was from a different bat genus than that from which HKU4 was isolated (Y. Yang *et al*. 2014). Additionally, unlike the MERS-CoV S protein, pseudoviruses containing the HKU4 S protein are able to infect a human cell line via DPP4, but only in the presence of exogenous trypsin, and to a lesser extent than pseudoviruses containing the MERS-CoV S protein. This is due to an inability of the human enzymes TMPRSS2 or endosomal proteases to cleave the bat HKU4 S proteins, although these host proteases effectively cleave the human MERS-CoV S protein (Wang *et al*. 2013; Y. Yang *et al*. 2014). By contrast, MERS-CoV is able to infect established bat cell lines expressing human DPP4 either endogenously or that are engineered to express it. Antibodies against human DPP4 were able to block viral cell entry

(Cai *et al*. 2014). Importantly, the cell lines used in this study were established from bats found in western Asia and northern Africa. Those cell lines able to be infected were from bat embryos, fetal lung and kidney, or adult kidney, but not from adult bat lung (Cai *et al*. 2014). This suggests that if human or dromedary MERS-CoV was indeed of bat origin, it may have been transmitted via the urinary, rather than the respiratory, route. Lung cells from *Rhinolophus landeri*, however, as well as kidney cells from *Roussetus aegyptiacus, P. pipistrellus, Myotis daubentonii*, and *Carollia perspicillata* bats are able to replicate MERS-CoV. These bat species represent four major chiropteran families from both bat suborders (Müller *et al*. 2012).

MERS-CoV can also infect cell lines from nonhuman primates, camels, civets, rabbits, goats, cattle, sheep, chickens, and pigs, but not cell lines of cat, dog, hamster, mouse, ferret, chicken, or insect origin (reviewed in Cai *et al*. 2014). Five amino acid variations in the MERS-CoV-binding domain of DPP4 from different species play a role in whether the host is susceptible or resistant to MERS-CoV infection (van Doremalen *et al*. 2014a). MERS-CoV-like antibodies have reported in dromedary camels in several countries having human MERS cases, but not in goats, sheep, cattle, horses, donkeys, or mules from the UAE and Spain (reviewed in van Doremalen *et al*. 2014b; Mackay & Arden 2015). The DPP4 protein from goats, cattle, and sheep are nevertheless still able to function as receptors for MERS-CoV, but with lower efficiency than for DPP4 from camels.

The complex structure by which bat HKU4's viral RBD binds DPP4 is similar to the binding mode used by human MERS-CoV (Wang *et al*. 2013), however, it lacks a helix and two small strands (b2 and b11) in the core subdomain as well as utilizing a 310 helix instead of the α-helix found in MERS-RBD (Wang *et al*. 2013). These key differences between HKU4 and MERS-CoV suggest that that the bat and human coronaviruses are quite distinct in their binding to the MERS-CoV receptor as well as their means of cleavage of the viral S protein. This suggests that changes in both of these processes need to occur before the bat HKU4 CoV can utilize human cells.

MERS-CoV is much more closely related to other bat coronaviruses than to HKU4 or HKU5. One of these is NeoCoV, the RNA of which was obtained directly from fecal material from a South African *N. capensis* bat (Corman *et al*. 2014). The genome consists of 30 100 nucleotides, with a G+C content of 40%, comparable with various MERS-CoV strains, whose genome is 30 100–30 107 nucleotides, with a G+C content of 41%. Amino acid sequence identity between NeoCoV and MERS-CoV strains in seven nested nonstructural protein domains was 97.2–97.4%, exceeding the 90% threshold used by the International Committee on Taxonomy of Viruses to define separate CoV species. Based upon taxonomic and other structural criteria, NeoCoV and MERS-CoV belong to a single viral species. Their S1 units are genetically divergent, suggesting that intraspike recombination events may have occurred during the emergence of MERS-CoV. NeoCoV is a sister taxon of MERS-CoV rooted between a novel African virus camel and all other viruses, suggesting that a higher level of viral diversity exists in camels than in humans and that camels were the source of virus in humans rather than vice versa. The majority of camels in the Arabian Peninsula are imported from the Greater Horn of Africa (Ethiopia, Sudan, Somalia, and Kenya), where several *Neoromicia* bat species also are found. This is an important point, since bats have only limited contact with humans in the Arabian Peninsula (noted in Khalafalla *et al*. 2015). The camels may have thus acquired MERS-CoV from these bats in Sub-Saharan Africa.

Dromedaries may thus have served as mixing vessels for MERS-CoV and other mammalian coronaviruses (Corman *et al*. 2014).

Another candidate for a MERS-CoV precursor from bats was found in a fecal pellet from a female *Neoromicia* cf. *zuluensis* collected in 2011. This beta-CoV (PML/2011) is closely related to MERS-CoV in a conserved 816-nucleotide fragment (1 amino acid difference; 0.3%). This is more closely related than a Ghana virus from *Nycteris* bats and the Chinese HKU4 and HKU5 bat coronaviruses previously discussed (5.5–7.7% amino acid difference). It is also more closely related to MERS-CoV than a 2c beta-CoV *RdRp* gene fragment from a Spanish *Hypsugo savii* bat, from a gene fragment from Thailand bat guano, and from a Mexican *Nyctinomops* bat in another, shorter, *RdRp* gene fragment (3.5–8.0% amino acid sequence difference). In fact, PML/2011 is as closely related by Bayesian phylogenetic analysis to MERS-CoV as bat CoV Rs672 is to SARS-CoV in this 816-nucleotide fragment. When a 269-nucleotide fragment from the 3'-terminus of the more variable *S* gene was studied, however, a 10.9% amino acid sequence distance was found between PML/2011 and MERS-CoV. A 13.3% difference in this region was also found between MERS-CoV and a European *Pipistrellus* CoV and a 20.5–27.3% difference between MERS-CoV and bat CoV HUK5 or HUK4 (Ithete *et al*. 2013). Coronaviruses from these bats, therefore, are not as closely related to MERS-CoV as NeoCoV.

The search for MERS-like coronaviruses is continuing in many areas of the world, with mixed results that are dependent, at least in part, upon whether or not the complete genomes are examined and, if not, which genes or gene products are tested and the length of the tested gene fragment. One should also keep in mind that some of the tested genes are highly conserved (*RdRp*), while others are more species-specific and are more relevant to host species tropism and ability to infect host target cells. One of these studies (Memish *et al*. 2013) collected feces and multiple tissue samples from 96 bats of 7 species with roosting sites in date palm orchards in close proximity to the index MERS case in Saudi Arabia. One of 29 tested *Taphozous perforatus* fecal pellets (3.5% infection rate) had 100% nucleotide sequence identity in a conserved *RdRp*190-nucleotide sequence to that of human beta-CoV RNA taken from the MERS index patient. MERS-related CoV RNA sequences have been amplified from members of the bat families Vespertillionidae, Molosidae, Nyteridae, and Emballonuridae (sheath-tailed bats) from Africa, the Americas, Asia, and Europe. It should be noted, however, that MERS itself in humans occurs, however, in very restricted areas of the world despite the detection of MERS-like viruses in bats over wide-spread regions.

In 2013, full-length genomic sequencing was performed on anal swab samples from *Vespertilio superans* from southwestern China (designated BtVs-BetaCoV/SC2013) The genome contains 30 143 nucleotides and has 75.7% nucleotide identity with human MERS-CoV. This is the greatest identity seen using full-length genomic analysis of bat sequences. This bat isolate also had 69.9% nucleotide identity with HKU4-1 and 70.1% identity with HKU5-1. Its S protein clusters in a clade with HKU5 and forms a superclade with HKU5, HKU4, and hCoV-MERS (Yang *et al*. 2014).

It has been suggested by several researchers (Guan *et al*. 2003; Ge *et al*. 2013; Reusken *et al*. 2013; Haagmans *et al*. 2014) that betacoronaviruses circulating in bats "jumped" to an intermediate host (civets and dromedary camels, in the cases of SARS-CoV and MERS-CoV, respectively) from which human infection occurred. If this is the case, it would be useful to determine the relationship between bat and civit or camel

isolates, particularly in the RMB, in order to test this hypothesis. Many other animal species also are infected with betacoronaviruses. It would be of value to also compare their complete RBD sequences with that of pathogenic human viruses in civits and camels, focusing efforts on those bats and other mammal species abundant in the region in which the disease originated.

5.3.5 Transmission of MERS-CoV

The patterns of spread of MERS-CoV among humans suggest that transmission occurs through droplets or contact. The DPP4 receptor expression differs in upper and lower respiratory tracts of humans. This may help to explain the observed human-to-human transmission which occurs more often in those who are immunocompromised or have comorbidities, such as diabetes (reviewed in Raj *et al*. 2014a). Interestingly, detailed population analysis demonstrates multiple MERS-CoV variants within single samples (quasispecies) may be present in individual dromedaries. In individual humans, however, only clonal genomic sequences have been found, suggesting that camel-to-human transmission may permit only specific genotypes capable of by-passing bottleneck selection (Briese *et al*. 2014). Increasing numbers of dromedaries and a recent trend towards locating herding operations near larger population areas may also increase human–camel contact (reviewed by Gossner *et al*. 2014).

Only a relatively small proportion of primary human cases, however, have had direct contact with dromedaries. Other routes of transmission include consumption of unpasteurized camel milk or raw meat or medicinal consumption of camel urine (Gossner *et al*. 2014). Camel milk consumption is becoming increasingly popular in the Arabian Peninsula, where cheese production is difficult and limited. In Saudi Arabia, 78% of the camel milk is unpasteurized, fresh, or fermented when sold to consumers. MERS-CoV has been isolated from camel milk samples, but it not known whether the virus is excreted in milk or if it was contaminated during milking or by an infected suckling calf (reviewed by Gossner *et al*. 2014). MERS-CoV injected into raw camel milk is stable upon refrigeration and infectious virus may be recovered even after storage for 2 days at room temperature, but is destroyed by heating to 63 °C for 30 min (van Doremalen *et al*. 2014a).

MERS-CoV has been detected in low concentration in human urine, so consumption of camel urine may be a risk factor, especially for those with underlying illness or immune deficiencies. Camel urine is customarily used to wash the hands, face, and hair among Bedouins and other camel-herding peoples in parts of the Middle East. Camel urine is also used in some traditional medical practices, such as treatment of gastrointestinal illness, to reduce blood clotting, as an anti-cancer agent, to strengthen the immune response, and to keep parasites out of the hair (reviewed in Abdel-Moneim 2014 and Mackay & Arden 2015). Fresh urine is drunk alone or combined with camel milk and is a component of some ointments and skin creams (reviewed by Gossner *et al*. 2014). Transmission via the eating of raw, contaminated meat is less likely, since normally meat is well-cooked, slaughtering is conducted hygienically, and the meat is chilled when sold commercially (reviewed in Abdel-Moneim 2014).

Distribution of primary cases of MERS is skewed towards older men in the Middle East, while it is fairly balanced among age and gender for secondary cases. This skewing of primary cases may be due to differential human exposure since camel rearing is

an exclusively male activity popular among middle-aged and retired men (Gossner *et al*. 2014). Greater susceptibility to and higher disease severity among those with comorbidities, including those in older age groups, may also be a factor.

MERS-CoV and SARS CoV remain viable for relatively long periods of time on surfaces. On plastic or steel, MERS-CoV remained viable for 8 h at 30 °C and 80% relative humidity, and for 24 h at 30 °C and 30% relative humidity. In aerosols, MERS-CoV viability decreased 89% at 70% relative humidity but only 7% at 40% relative humidity at 20 °C. MERS-CoV survival is less than that of SARS-CoV, however it may thoroughly contaminate a room occupied by a symptomatic patient (reviewed by Mackay & Arden 2015). This should call attention to the risks associated with transmission of MERS-CoV and SARS-CoV by bioaerosols in settings such as waiting, treatment, and patient rooms; emergency departments; and open intensive care facilities. The quality of air exchange, circulation, and filtration; use of proper infection control procedures; and personnel protective care are important factors in risk reduction, particularly in light of the growing numbers of human-to-human hospital-based transmissions in Saudi Arabia and that which occurred in South Korea.

Bats may also indirectly transmit infection to humans. One index case lived and worked in close proximity to an abandoned date palm orchard. Roosting bats and guano was present in abandoned wells and ruins of the area. Food or water of domestic animals, including dromedaries, in areas containing palm orchards may be contaminated with bat guano, saliva, and/or urine, infecting the camels, and leading to human infection (reviewed in Abdel-Moneim 2014). This hypothesis bears testing in areas in where bats and dromedaries cohabit.

5.4 OTHER CORONAVIRUSES OF BATS

The contention that bats may act as a major reservoir of alpha- and betacoronaviruses is supported by the fact that their genetic diversity is greater in bats than is currently known for any other host (Drexler *et al*. 2014). Even though coronaviruses are found in bat feces or urine, they cause no apparent gastrointestinal or other disease symptoms in these hosts, perhaps due to their high level of anti-CoV antibody generation (Drexler *et al*. 2014). Persistence of viruses in bat populations appears to rely on massive amplification during bat reproductive cycles, possibly due to fecal–oral transmission, as seen with other viruses, such as filoviruses, henipaviruses, astroviruses, and lyssaviruses in bat populations (Drexler *et al*. 2014).

In addition to those CoV species discussed previously, human coronavirus HCoV-229E and coronaviruses from Ghanaian *Hipposideros* bats share common ancestry (reviewed in Reusken *et al*. 2013). Further work to examine the extent of diversity of coronaviruses in other groups of mammals, especially in China and the Arabian Peninsula, is required, especially since coronaviruses infect mice and mice are known reservoirs for another severe respiratory illness caused by hantaviruses. A distinct lineage c beta-CoV (EriCoV) has been identified in hedgehogs. Human CoV HCoV-OC43 also has recent common ancestry with bovine CoV (reviewed in Reusken *et al*. 2013).

A 2013 study amplified regions of RNA encoding the helicase, S, and capsid or envelope proteins from 96 bats of 7 species with roosting sites in date palm orchards in close proximity to the index MERS case in Saudi Arabia. Of note, the chosen RNA

regions detected both alpha-CoV and beta-CoV RNA sequences even though this test was believed to be a MERS-CoV-specific assay and MERS-CoV is a beta-CoV. In this study, both alpha-CoV and beta-CoV RNA were amplified from insectivorous *T. perforatus* and *R. hardwickii* and the frugivorous *E. helvum* bats, but only alpha-CoV RNA was amplified from the insectivorous *P. kuhlii*. CoV RNA was present in bat rectal swabs and 23% of the fecal pellets and roost feces, with alpha-CoV detected more often than the beta-CoV group to which human SARS-CoV and MERS-CoV belong. No CoV RNA was found in throat swabs or urine or serum samples, suggesting that transmission between animals occurs via contact with infected fecal material (Memish *et al*. 2013).

Samples were collected from anal swabs of 75 insectivorous Italian Vespertillionidae bats (*Myotis myotis, M. blythii, Eptesicus serotinus*) in northern Italy after bat reproduction during the summers of 2008–2012. Two novel alphacoronaviruses were detected from *M. blythii* as well as two new lineage c betacoronaviruses from *E. serotinus* (ITA31/384/2012). Using nested RT-PCR, the betacoronaviruses were found to have 96.9% predicted amino acid sequence homology in a 816-nucleotide fragment of the conserved *RdRp* gene and to cluster with bat CoV from Spanish *E. isabellinus* (also found in the northern Sahara). The new alpha-CoV clusters with Spanish bat CoV from *M. blythii* and *Miniopterus schreibersi*, as well as *Myotis dasycneme* from the Netherlands (De Benedictis *et al*. 2014). Five distinct alpha-CoV clades were isolated from rectal swabs of *Rhinolophus* and *Myotis* species from Yunnan, China, as well (He *et al*. 2014). Several other studies found SARS-like CoV in several insectivorous bat species in China, Europe, and Africa that have 76–78% nucleotide identity in variable *S* gene and a 19-amino acid deletion in the RBD. Previous reports found coronaviruses in 20 bat species from four families throughout China and Hong Kong: 10 species from Vespertillionidae, 8 from Rhinolophidae, 1 from Molossidae, and 1 from Pteropodidae (reviewed in He *et al*. 2014). Muluccan naked-backed fruit bat (*Dobsonia moluccensisi*) from Indonesia harbors a beta-CoV RNA in 4.1% of tested fecal samples ($n=74$) (Anindita *et al*. 2015). This virus is most closely related to BatCoV HKU9 from China and BatCoV KY06 from Kenya. The large number and diversity of beta-CoV isolates from bats, the relative lack of knowledge of CoV diversity in other mammal species, the lack of some ORF, presence of nucleotide deletions in critical regions, and the wide range of nucleotide and amino acid identity in the RBD make it difficult to know which bat CoV served as a predecessor to either SARS-CoV or MERS-CoV or whether the CoV predecessor originated in a different group of mammals. Further research should help to uncover the history of the pathogenic human coronaviruses and perhaps the likelihood that bat-to-human zoonotic transfer will happen again.

A study of feces from multiple bat species inhabiting an abandoned mineshaft in China ($n=256$) found CoV RNA in feces from all of the following species: *R. sinicus, R. affinis, Hipposideros pomona, M. schreibersi, Miniopterus fuliginosus*, and *Miniopterus fuscus* (Ge *et al*. 2016). Prevalence of infection among the bat species ranged from 45 to 74%. Almost all of the viral sequences were related to previously known alphaviruses: HKU1 was present in *R. sinicus, M. schreibersi, M. fuliginosus*; HKU2 in *R. sinicus* and *R. affinis*; HKU7 in *M. schreibersi*; HKU8 in *R. sinicus, R. affinis, M. schreibersi*, and *M. fuscus*; and HKU10 in *H. pomona*. A novel SARS-like beta-CoV (RaBtCoV/4991) was also detected in *R. affinis* in addition to a novel beta-CoV (HpBtCoV/3740-2) in *H. pomona*. Co-infection with several CoV species occurred in all six of these bat species, a situation that increases the chance of recombination (Ge *et al*. 2016).

5.5 CONCLUSIONS

Coronaviruses are large, enveloped, ssRNA (+) viruses that infect many mammals and birds. Alpha- and betacoronaviruses contain members that cause mild to life-threatening respiratory, enteric, hepatic, or neurological disease in humans. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU typically cause mild cold-like symptoms in immunocompetent humans, however, the SARS-CoV and MERS-CoV betacoronaviruses cause severe respiratory disease with high mortality rates. SARS-CoV, civet SARS-related coronaviruses, and SARS-related *Rhinolophus* bat coronaviruses are from beta-CoV lineage b, while MERS-CoV, and HKU4 and HKU5 bat coronaviruses are from lineage c. The following discussion will focus on betacoronaviruses from lineages b and c.

Genetic diversity in coronaviruses is partially due to the infidelity of its polymerase and their atypically large genome. This diversity may allow accumulation of novel traits which equip viral progeny to exploit different ecological niches and hosts, leading to interspecies transmission as may have occurred with HCoV-OC43, a cattle CoV that may have entered humans via zoonotic transmission.

Eleven bat families (the vast majority being insectivorous) contain species that either been exposed to or infected by alpha- or betacoronaviruses. Two of the four frugivorous bat species associated with a SARS-like CoV are restricted to Madagascar, while SARS originated in China. SARS-CoV is known to be transmitted to humans by close contact with several species of live animals from Chinese wet-markets, including palm civits. Civits are claimed to been infected by Chinese fruit bats, however, only insectivorous bat species harbor SARS-like coronaviruses in Asia or Southeast Asia.

Host species and host cellular targets result, to a large degree, from interactions between the viral S protein, responsible for receptor binding and fusion, and the host cell receptor, ACE-2 for SARS-CoV. Sequence identity of the *S* genes of bat and human or civit isolates is 76–78%, and that of the critical S1 domain is only 63–64%. Of note, bat isolates also have a six amino acid insertion and three deletions in S1, several of these found in the RBD.

SARS-CoV is well-adapted to the human ACE2 receptor and is unable to infect bat cells or bind ACE2 from most bats. Bat ACE2 and human ACE2 have amino acid identity of 80–82%, which may contribute to the failure of SARS-CoV to infect bat cells. By contrast, civit and human ACE2 differ by only two amino acids. A human SARS-CoV isolate grew similarly in cells expressing either human or civit ACE2.

Whole-genome sequencing discovered two novel bat coronaviruses (RsSHC014 and Rs3367) whose genes have a high degree of homology with the RBD of SARS-CoV's S protein. One or both of these isolates can use human ACE2 for cell entry, making them better candidates for a predecessor to SARS-CoV than other bat coronaviruses. Full-genome sequencing of human and palm civit SARS-CoV isolates, however, revealed 99.8% homology, much higher than that seen for bat SARS-like CoV.

MERS originated in and is confined primary to the Middle East. The host cell receptor for the MERS-CoV S protein is DPP4, which is conserved among many animal species, including human and nonhuman primates, dromedaries, sheep, cows, and bats. Zoonotic transmission of MERS-CoV to humans is via nasal secretions of dromedaries, drinking their raw milk or urine, and human-to-human. One human MERS-CoV isolate

was identical to that of a sick dromedary with which the human had close contact, further strengthening the ties between human and dromedary MERS-CoV.

Two bat MERS-like coronaviruses, HKU4 and HKU5, have been suggested to be linked to human infections. However, they have very low (40–55%) identity to the human MERS-CoV RBD and HKU5 also contains deletions in this region. This evidence strongly suggests that these bat viruses are unlikely to be responsible for transmission to humans. Since bat kidneys and urine are infected with these coronaviruses, transmission to humans, if it were to occur, would be via bat urine.

MERS-CoV is much more closely related to NeoCoV from fecal material of a South African bat (Corman *et al*. 2014). Amino acid identity between the bat and human viruses for seven proteins was approximately 97% and taxonomic criteria suggest that NeoCoV and MERS-CoV are a single viral species. It should be noted that the presence of viral RNA or proteins in feces does not necessarily mean that the bats were infected, since the viruses may instead have merely passed through the animals' digestive tracts. A number of other studies found varying degrees of nucleotide homology or identity between human MERS-CoV and various bat coronaviruses using relatively small fragments of conserved genes. The fact that these bats were from locations throughout the world and that human MERS is acquired in very restricted areas of the world would suggest that there is little risk of zoonotic transmission from bats and that research efforts perhaps should focus to a greater degree on dromedaries, which are known to transmit MERS-CoV to humans.

REFERENCES

Abdel-Moneim AS. 2014. Middle East respiratory syndrome coronavirus (MERS-CoV): evidence and speculations. *Archives of Virology.* 159:1575–1584.

Adney DR, van Doremalen N, Brown VR, Bushmaker T, Scott D, de Wit E, Bowen RA, Munster VJ. 2014. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerging Infectious Diseases.* 20(12):1999–2005.

Anindita PD, Sasaki M, Setiyono A, Handharyani E, Orba Y, Kobayashi S, Rahmadani I, Taha S, Adiani S, Subangkit M, Nakamura I, Sawa H, Kimura T. 2015. Detection of coronavirus genomes in Moluccan naked-backed fruit bats in Indonesia. *Archives of Virology.* 160:1113–1118.

Azhar EI, El-Kafrawy SA, Farraj SA, Hassan AM, Al-Saeed MS, Hashem AM, Mladani TA. 2014. Evidence for camel-to-human transmission of MERS coronavirus. *New England Journal of Medicine.* 370:2499–2505.

Briese T, Mishra N, Jain K, Zalmout IS, Jabado OJ, Karesh WB, Daszak P, Mohammed OB, Alagaili AN, Lipkin WI. 2014. Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. *mBio*. 5(3):e01146–14.

Cai Y, Yú S, Postnikova EN, Mazur S, Bernbaum JG, Burk R, Zhāng T, Radoshitzky SR, Müller MA, Jordan I, Bollinger L, Hensley LE, Jahrling PB, Kuhn JH. 2014. CD26/DPP4 cell-surface expression in bat cells correlates with bat cell susceptibility to Middle East respiratory syndrome coronavirus (MERS-CoV) infection and evolution of persistent infection. *PLoS ONE.* 9(11):e112060.

Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ. 2008. Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Research.* 133:13–19.

Chan SMS, Damdinjav B, Perera RAPM, Chu DKW, Khishgee B, Enkhbold B, Poon LLM, Peiris M. 2015. Absence of MERS-coronavirus in Bactrian camels, Southern Mongolia, November 2014. *Emerging Infectious Diseases*. 21(7)1:269–1271.

Corman VM, Ithete NL, Richards LR, Schoeman MC, Preiser W, Drosten C, Drexler JF. 2014. Rooting the phylogenetic tree of Middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat. *Journal of Virology.* 88(19):11297–11303.

De Benedictis P, Marciano S, Scaravelli D, Priori P, Zecchin B, Capua I, Monnne I, Cattoli G. 2014. Alpha and lineage C betaCoV infections in Italian bats. *Viral Genes.* 48:366–371.

Denison MR, Graham RL, Donaldson EF, Eckerle LD, Baric RS. 2011. Coronaviruses: An RNA proofreading machine regulates replication fidelity and diversity. *RNA Biology.* 8(2):270–279.

Drexler JF, Corman VM, Drosten C. 2014. Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research.* 101:45–56.

Eckerle I, Corman VM, Müller MA, Lenk M, Ulrich RG, Drosten C. 2014. Replicative capacity of MERS coronavirus in livestock cell lines. *Emerging Infectious Diseases.* 20(2):276–279.

Ge XY, Li JL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W, Peng C, Shag YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF, Daszak P, Shi ZL. 2013. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature.* 503(7477):535–538.

Ge XY, Wang N, Zhang W, Hu B, Li B, Zhang YZ, Zhou JH, Luo CM, Yang XL, Wu LJ, Wang B, Zhang Y, Li ZX, Shi ZL. 2016. Coexistence of multiple coronaviruses in several bat colonies in an abandoned mineshaft. *Virologica Sinica.* 31(1):31–40.

Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, Luo SW, Li PH, Zhang LJ, Guan YJ, Butt KM, Wong KL, Chan KW, Lim W, Shortridge KF, Yuen KY, Peiris JS, Poon LL. 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science*. 302:276–278.

Guo Y, Korteweg C, McNutt MA, Gu J. 2008. Pathogenic mechanisms of severe acute respiratory syndrome. *Virus Research*. 33:4–12.

Gossner C, Danielson N, Gervelmeyer A, Berthe F, Faye B, Aaslav KK, Adlhoch C, Zeller H, Penttinen P, Coulombier D. 2014. Human–Dromedary camel interactions and the risk of acquiring zoonotic Middle East respiratory syndrome coronavirus infection. *Zoonoses and Public Health*. 63(1):1–9.

Gouilh MA, Puechmaille SJ, Gonzalez J-P, Teeling E, Kittayapong P, Manuguerra J-C. 2011. SARS-Coronavirus ancestor's foot-prints South-East Asian bat colonies and the refuge theory. *Infection, Genetics and Evolution*. 11:1690–1702.

Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, Godeke GJ, Jonges M, Farag E, Diab A, Ghobashy H, Alhajri F, Al-Thani M, Al-Marri SA, Al Romaihi HE, Al Khal A, Bermingham A, Osterhaus AD, AlHajri MM, Koopmans MP. 2014. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infectious Diseases*. 14:140–145.

He B, Zhang Y, Xu L, Yang W, Yang F, Feng Y, Xia L, Zhou J, Zhen W, Feng Y, Guo H, Zhang H, Tu C. 2014. Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome-like coronavirus from bats in China. *Journal of Virology.* 88(12):7070–7082.

Hilgenfeld R, Peiris M. 2013. From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral Research*. 100:286–295.

Hou Y, Peng C, Yu M, Li Y, Han Z, Li F, Wang L-F, Shi Z. 2010. Angiotensin-converting enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to SARS-CoV entry. *Archives of Virology*. 155:1563–1569.

Ithete NL, Sroffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, Drosten C, Drexler JF, Preiser W. 2013. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. *Emerging Infectious Diseases*. 19(10):1697–1699.

Khalafalla AI, Lu X, Al-Mubarak AIA, Dalab AHS, Al-Busadah KAS, Erdman DD. 2015. MERS-CoV in upper respiratory tract and lungs of dromedary camels, Saudi Arabia, 2013–2014. *Emerging Infectious Diseases*. 21(7):1153–1158.

Kim HK, Yoon S-W, Kim D-J, Koo B-S, Noh JY, Kim JH, Choi YG, Na W, Chang K-T, Song D, Jeong DG. 2016. Detection of severe acute respiratory syndrome-like, Middle East respiratory syndrome-like bat coronaviruses and group H rotavirus in faeces of Korean bats. *Transboundary and Emerging Infections*. DOI:10.1111/tbed.12515.

Lau SK, Li KS, Huang Y, Shek CT, Tse H, Wang M, Choi GK, Xu H, Lam CS, Guo R, Chan KH, Zheng BJ, Woo PC, Yuen KY. 2010a. Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related *Rhinolophus* bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. *Journal of Virology*. 84(6):2808–2819.

Lau SK, Poon RW, Wong BH, Wang M, Huang Y, Xu H, Guo R, Li KS, Gao K, Chan KH, Zheng BJ, Woo PC, Yuen KY. 2010b. Coexistence of different genotypes in the same bat and serological characterization of *Rousettus* bat coronavirus HKU9 belonging to a novel Betacoronavirus subgroup. *Journal of Virology*. 84(21):11385–1194.

Lau SKP, Li KSM, Tsang AKL, Lam CSF, Ahmed S, Chen H, Chan K-H, Woo PCY, Yuen K-Y. 2013. Genetic characterization of *Betacoronavirus* lineage c viruses in bats reveals marked sequence divergence in the spike protein of *Pipistrellus* bat coronavirus HKU5 in Japanese pipistrelle: implications for the origin of the novel Middle East respiratory syndrome coronavirus. *Journal of Virology*. 87(15):8638–8650.

Liu L, Fang Q, Deng F, Wang H, Yi CE, Ba L, Yu W, Lin RD, Li T, Hu Z, Ho DD, Zhang L, Chen Z. 2007. Natural mutations in the receptor binding domain of spike glycoprotein determine the reactivity of cross-neutralization between palm civet coronavirus and severe acute respiratory syndrome coronavirus. *Journal of Virology*. 81(9):4694–4700.

Lu G, Hu Y, Wang Q, Qi J, Gao F, Li Y, Zhang Y, Zhang W, Yuan Y, Bao J, Zhang B, Shi Y, Yan J, Gao GF. 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature*. 500: 227–231.

Mackay IM, Arden KE. 2015. Middle East respiratory syndrome: An emerging coronavirus infection tracked by the crowd. *Virus Research*. 202:60–88.

Memish ZA, Mishra N, Olivai KJ, Fagbo SF, Kapoor V, Epstein JH, AlHakeem R, Al Asmari M, Islam A, Kapoor A, Breise T, Daszak P, Al Rabeeah AA, Lipkin WI. 2013. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerging Infectious Diseases*. 19(11):1819–1823.

Müller MA, Corman VM, Jores J, Meyer B, Younan M, Liljander A, Bosch B-J, Lattwein E, Hilali M, Musa BE, Bornstein S, Drosten C. 2014. MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983–1997. *Emerging Infectious Diseases*. 20(12): 2093–2095.

Müller MA, Raj VS, Muth D, Meyer B, Kallies S, Smits SL, Wollny R, Bestebroer TM, Specht S, Suliman T, Zimmermann K, Binger T, Eckerle I, Tschapka M, Zaki AM, Osterhaus ADME, Fouchier FAM, Haagmans BL, Drosten C. 2012. Human coronavirus EMC does not require the SARS-coronavirus receptor and maintains broad replicative capability in mammalian cell lines. *mBio*. 3(6):e00515–12.

Perera R, Wang P, Gomaa M, El–Shesheny R, Kandeil A, Bagato O, Siu L, Shehata M, Kayed A, Moatasim Y, Li M, Poon LL, Guan Y, Webby RJ, Ali MA, Peiris JS, Kayali G. 2013. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus

neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. *European Surveillance.* 18:20574.

Raj VS, Osterhaus ADME, Fouchier RAF, Haagmans BL. 2014a. MERS: emergence of a novel human coronavirus. *Current Opinions in Virology.* 5:58–62.

Raj VS, Smits SL, Provacia LB, van den Brand JMA, Wiersma L, Ouwendijk WJD, Bestebroer TM, Spronken MI, van Amerongen G, Rottier PJM, Fouchier RAM, Bosch BJ, Osterhaus ADME, Haagmans BL. 2014b. Adenosine deaminase acts as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the Middle East respiratory syndrome coronavirus. *Journal of Virology*. 88(3):1834–1838.

Razanajatovo NH, Nomenjanahary LA, Wilkinson DA, Razafimanahaka JH, Goodman SM, Jenkins RK, Jones JPG, Heraud J-M. 2015. Detection of new genetic variants of betacoronaviruses in endemic frugivorous bats of Madagascar. *Virology Journal.* 12:42.

Ren W, Li W, Yu M, Hao P, Zhang Y, Zhou P, Zhang S, Zhao G, Zhong Y, Wang S, Wang LF, Shi Z. 2006. Full-length genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis. *Journal of General Virology.* 87:3355–3359.

Ren W, Qu X, Li W, Han Z, Yu M, Zhou P, Zhang S-Y, Wang L-F, Deng H, Shi Z. 2008. Difference in receptor usage between severe acute respiratory syndrome (SARS) coronavirus and SARS-like coronavirus of bat origin. *Journal of Virology*, 82(4):1899–1907.

Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, Muth D, Raj VS, Smits-De Vries L, Corman VM, Drexler JF, Smits SL, El Tahir YE, De Sousa R, van Beek J, Nowotny N, van Maanen K, Hidalgo-Hermoso E, Bosch BJ, Rottier P, Osterhaus A, Gortázar-Schmidt C, Drosten C, Koopmans MP. 2013. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infectious Diseases.* 13:859–866.

Reusken CBEM, Messadi L, Feyisa A, Ularamu H, Godeke G-J, Danmarwa A, Dawo F, Jemli M, Melaku S, Shamaki D, Woma Y, Wungak Y, Gebremedhin EZ, Zutt I, Bosch B-J, Haagmans BL, Koopmans MPG. 2014. Geographic distribution of MERS coronavirus among dromedary camels, Africa. *Emerging Infectious Diseases.* 20(8):1370–1374.

Sheahan T, Rockx B, Donaldson E, Corti D, Baric R. 2008. Pathways of cross-species transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome coronavirus. *Journal of Virology.* 82(17):8721–8732.

Tan Y-J, Lim SG, Hong W. 2006. Understanding the accessory viral proteins unique to the severe acute respiratory syndrome (SARS) coronavirus. *Antiviral Research.* 72:78–88.

Thiel V, Weber F. 2008. Interferon and cytokine responses to SARS-coronavirus infection. *Cytokine & Growth Factor Reviews.* 19:121–132.

van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus ADME, Haagmans BL, Gorbalenya AE, Snijder EJ, Fouchier RAM. 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio*. 3(6):e00473–12.

van Doremalen N, Bushmaker T, Karesh WB, Munster VJ. 2014a. Stability of Middle East respiratory syndrome coronavirus in milk. *Emerging Infectious Diseases.* 20(7):1263–1264.

van Doremalen N, Miazgowicz KL, Milne-Price S, Bushmaker T, Robertson S, Scott D, Kinne J, McLellan JS, Zhu J, Munster VJ. 2014b. Host species restriction of Middle East respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4. *Journal of Virology.* 88(16):9220–9232.

Wang N, Shi X, Jiang L, Zhang S, Wang D, Tong P, Guo D, Fu L, Cui Y, Liu X, Arledge KC, Chen Y-H, Zhang L, Wang X. 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Research.* 23:986–993.

Wernery U, Corman VM, Wong EYM, Tsang AKL, Muth D, Lau SKP, Khazanehdari K, Zirkel F, Ali M, Nagy P, Juhasz J, Wernery R, Joseph S, Syriac G, Elizabeth SK, Patteril NAG, Woo PCY, Drosten C. 2015. Acute Middle East respiratory syndrome coronavirus infection in livestock dromedaries, Dubai, 2014. *Emerging Infectious Diseases.* 21(6):1019–1022.

Woo PCY, Lau SKP, Li KSM, Tsang AKL, Yuen K-Y. 2012. Genetic relatedness of the novel human group c betacoronavirus to *Tylonycteris* bat coronavirus HKU4 and *Pipistrellus* bat coronavirus HKU5. *Emerging Microbes and Infections.* 1:e35.

Yang L, Wu Z, Ren F, Zhang J, He G, Dong J, Sun L, Zhu Y, Zhang S, Jin Q. 2014. MERS-related betacoronavirus in *Vespertilio superans* bats, China. *Emerging Infectious Diseases.* 20(7): 1260–1262.

Yang Y, Du L, Liu C, Wang L, Ma C, Tang J, Baric RS, Jiang S, Li F. 2014. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proceedings of the National Academy of Sciences USA*. 111(34):12516–12521.

Yuan J, Hon C-C, Li Y, Wang D, Xu G, Zhang H, Zhou P, Poon LLM, Lam TT-Y, Leung FC-C, Shi Z. 2010. Intraspecies diversity of SARS-like coronaviruses in *Rhinolophus sinicus* and its implications for the origin of SARS coronaviruses in humans. *Journal of General Virology.* 91:1058–1062.

Zhou P Li H, Wang H, Wang LF, Shi Z. 2012. Bat severe acute respiratory syndrome-like coronavirus ORF3b homologues display different interferon antagonist activities. *Journal of General Virology.* 93:275–281.

6

OTHER RNA VIRUSES AND BATS

6.1 INTRODUCTION

Four classes of viruses use RNA as their genetic information: Baltimore Class III (double-stranded RNA), Baltimore Class IV (single-stranded, positive RNA), Baltimore Class V (single-stranded, negative RNA), and Baltimore Class VI (reverse-transcribing RNA viruses). This chapter will discuss the interactions between bats and Classes III–V RNA viruses, with the exception of the following viral families which are the subjects of separate chapters: Coronaviridae, Filoviridae, Paramyxoviridae, and Rhabdoviridae. Class VI viruses will also be discussed in another chapter as well. Classes III–V use the enzyme RNA-dependent RNA polymerase during part of their lifecycle. This enzyme is very error-prone; thus these classes of viruses generally have a higher mutation rate than that of DNA viruses.

6.2 BALTIMORE CLASS III VIRUSES AND BATS

Baltimore Class III RNA viruses include reoviruses (respiratory enteric orphan viruses). They were discovered in the 1950s and, since they were not known to be associated with human disease at that time, were designed to be "orphan viruses." Reoviridae is a diverse family of non-enveloped viruses with segmented dsRNA genomes. It is genetically divided into the Sedoreovirinae subfamily with six genera and the Spinareovirinae subfamily with nine genera. The Sedoreovirinae subfamily includes orbiviruses and rotaviruses. The Spinareovirinae subfamily contains the *Orthoreovirus* genus that

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presently contains five species: *Pteropine orthoreovirus, Avian orthoreovirus, Reptilian orthoreovirus, Baboon orthoreovirus,* and *Mammalian orthoreovirus*. *Pteropine orthoreovirus* contains orthoreoviruses of bats and *Mammalian orthoreovirus* includes orthoreoviruses of humans and most mammals, including some viruses of bats (reviewed by Kohl *et al*. 2012). See Table 6.1 for a list of a variety of RNA viruses associated with bats. Members of the genus *Orthoreovirus* contain 10 genomic segments. They infect a wide variety of mammals, birds, reptiles, fish, insects, and plants. The five members of this genus may be divided into two groups: fusogenic and nonfusogenic. Fusogenic reoviruses induce cell–cell fusion and syncytium formation (reviewed by Chua *et al*. 2008). Only the *Mammalian orthoreovirus* species are nonfusogenic. Limited sequence conservation occurs between different genera of reoviruses and members have distinct capsid morphologies, host ranges, replication strategies, and protein profiles. Sequence analysis revealed significant divergence among fusogenic, as well as between fusogenic and nonfusogenic, orthoreoviruses (reviewed by Pritchard *et al*. 2006).

6.2.1 Orbiviruses

Eidolon helvum from Africa were found to harbor an orbivirus, Ife virus. Japanaut virus is another orbivirus detected in the long-tongued fruit bat (*Syconycteris crassa*) in Papua New Guinea, while Fomédé virus was found in the Gambian slit-faced bat (*Nycteris gambiensis*) and the dwarf slit-faced bat (*Nycteris nana*) (reviewed in Kohl & Kurth 2015).

6.2.2 Rotaviruses

Bat rotavirus A was detected by PCR in *E. helvum* in Kenya. Another bat rotavirus A strain, related to canine and feline strains, was isolated from *Rhinolophus hipposideros* from China (reviewed by Kohl & Kurth 2015). Rotaviruses cause diarrhea in infants and young children that may be life-threatening.

6.2.3 Pteropine orthomyxovirus group

Nelson Bay virus was isolated in 1968 from the heart blood of the gray-headed flying fox (*Pteropus poliocephalus*) in Australia (Gard & Compans 1970). It was the first known fusogenic mammalian reovirus; this property had been thought to be restricted to avian reoviruses. While having a typical reovirus structure, Nelson Bay virus is "strikingly different from mammalian reoviruses." Nelson Bay virus causes a rapid cytopathic effect with syncytia containing twenty or more nuclei in a pig kidney cell line. Cytoplasmic vacuolization occurs and nuclei at the margins of the syncytia have a web-like appearance while the nuclei in the syncytia's center degenerate. Upon intracerebral inoculation of infant mice, the virus causes paralysis and spasticity prior to death (Gard & Compans 1970). A new orthoreovirus from Tioman Island in Malaysia, Pulau virus, was subsequently isolated from urine samples from the variable flying fox (*Pteropus hypomelanus*) after culture in Vero kidney cells, in which it forms large syncytia (Pritchard *et al*. 2006). The prevalence of seropositivity in humans for Pulau virus from bats and the closely related human Melaka virus

TABLE 6.1 Assorted RNA viruses associated with bats

Bat family	Bat common name	Bat species	Virus
Phyllostomidae	Tailed tailless bat	*Anoura caudifer*	Araraquara virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	Bimiti virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	Catu virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	Dengue 2 virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	Guama virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	Ilheus virus
Phyllostomidae	Geoffroy's tailless Bat	*Anoura geoffroyi*	St. Louis encephalitis virus
Phyllostomidae	Gervais's fruit-eating bat	*Artibeus cinereus*	Dengue 2 virus
Phyllostomidae	Gervais's fruit-eating bat	*Artibeus cinereus*	Ilheus virus
Phyllostomidae	Gervais's fruit-eating bat	*Artibeus cinereus*	Yellow fever virus
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	Dengue 1
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	Dengue 2
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	Dengue 4
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Eastern equine encephalitis virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Ilheus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Nepuyo virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Restan virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	St. Louis encephalitis virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Tacaribe virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Venezuelan equine encephalitis virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Vesicular stomatitis virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	West Nile virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Western equine encephalitis virus
Phyllostomidae	Jamaican fruit-eating bat	*Artebius jamaicensis*	Yellow fever virus

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Caraparu virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Dengue 1
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Dengue 2
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Dengue 4
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Eastern equine encephalitis virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Ilheus virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Influenza A H18N11
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Nepuyo virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Oriboca virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Restan virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	St. Louis encephalitis virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Tacaribe virus
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	Yellow fever virus
Phyllostomidae	Dark Fruit-eating Bat	*Artibeus obscurus*	Influenza A virus H18N11
Phyllostomidae	Pygmy Fruit-eating Bat	*Artibeus phaeotis*	St. Louis encephalitis virus
Phyllostomidae	Pygmy Fruit-eating Bat	*Artibeus phaeotis*	Venezuelan equine encephalitis virus
Phyllostomidae	Pygmy Fruit-eating Bat	*Artibeus phaeotis*	Vesicular stomatitis virus
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	Influenza A virus H18N11
Phyllostomidae	Fruit-eating bats	*Artebius* sp.	Tamana bat virus
Phyllostomidae	Teapa fruit-eating bat	*Artibeus turpis*	Venezuelan equine encephalitis virus
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	Dengue virus
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	Influenza A virus H18N11
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	Venezuelan equine encephalitis virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Bimiti virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Catu virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Dengue 2 virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Eastern equine encephalitis virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Ilheus virus

Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Influenza A virus H18N11
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Restan virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	St. Louis encephalitis virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Tamana bat virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Venezuelan equine encephalitis virus
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	Yellow fever virus
Phyllostomidae	Gray short-tailed bat	*Carollia subrufa*	Venezuelan equine encephalitis virus
Vespertilionidae	Wrinkle-lipped free-tailed bat	*Chaerephon plicata*	Kaeng Khoi
Vespertilionidae	Little free-tailed bat	*Chaerephon pumilus*	Chikungunya virus
Vespertilionidae	Little free-tailed bat	*Chaerephon pumilus*	Dakar bat virus
Vespertilionidae	Little free-tailed bat	*Chaerephon pumilus*	Entebbe bat salivary gland virus
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Carey Island virus
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Japanese encephalitis virus
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Jugra virus
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Phnom-Penh bat virus
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx*	Kyasanur Forest disease virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Influenza A virus H18N11
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Tacaribe virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Tamana bat virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Venezuelan equine encephalitis virus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Vesicular stomatitis virus
Phyllostomidae	Hairy-legged vampire bat	*Diphylla ecaudata*	Araraquara virus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Bat rotavirus A
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Ife virus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Kumasi rhabdovirus
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	Phnom-Penh bat virus
Pteropodidae	Epauletted fruit bats	*Epomophorus* sp.	Yellow fever virus
Pteropodidae	Franquet's epauletted fruit bat	*Epomops franqueti*	Rift Valley fever virus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Rio Bravo virus

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Saint Louis encephalitis virus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	West Nile virus
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Astrovirus sp.
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Hantaan virus
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Hepevirus
Vespertilionidae	None	*Eptesicus* sp.	Eastern encephalitis virus
Vespertilionidae	None	*Eptesicus* sp.	Western encephalitis virus
Rhinolophidae	Great roundleaf bat	*Hipposideros armiger*	Astrovirus sp.
Rhinolophidae	Pomona roundleaf bat	*Hipposideros pomona*	Bat sapovirus
Vespertilionidae	Silvered bat	*Glauconycteris argentata*	Rift Valley fever virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Dengue 1
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Dengue 2
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Dengue 4
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Eastern equine encephalitis virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Ilheus virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	St. Louis encephalitis virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Tamana bat virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Venezuelan equine encephalitis virus
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Yellow fever virus
Phyllostomidae	Aba roundleaf bat	*Hipposideros abae*	Hepevirus
Phyllostomidae	Aba roundleaf bat	*Hipposideros abae*	Rift Valley fever virus
Phyllostomidae	Noack's roundleaf bat	*Hipposideros cf. ruber*	Hepevirus
Rhinolophidae	Great roundleaf bat	*Hipposideros armiger*	Japanese encephalitis virus
Rhinolophidae	Great roundleaf bat	*Hipposideros armiger*	Picornavirus sp., group 3
Rhinolophidae	Bicolored roundleaf bat	*Hipposideros bicolor*	Japanese encephalitis virus
Rhinolophidae	Sundevall's roundleaf bat	*Hipposideros caffer*	Chikungunya virus
Rhinolophidae	Sundevall's roundleaf bat	*Hipposideros caffer*	Rift Valley fever virus
Rhinolophidae	Ashy roundleaf bat	*Hipposideros cineraceus*	Japanese encephalitis virus

Rhinolophidae	Jones' roundleaf bat	*Hipposideros jonesi*	Kolente virus
Rhinolophidae	Giant roundleaf bat	*Hipposideros gigas*	Leopards Hill virus
Rhinolophidae	Intermediate leaf-nosed bat	*Hipposideros larvatus*	Astrovirus sp.
Rhinolophidae	Pomona leaf-nosed bat	*Hipposideros pomona*	Astrovirus sp.
Rhinolophidae	Pomona leaf-nosed bat	*Hipposideros pomona*	Hantavirus
Rhinolophidae	Pomona leaf-nosed bat	*Hipposideros pomona*	Japanese encephalitis virus
Rhinolophidae	Pomona leaf-nosed bat	*Hipposideros pomona*	Xuan son virus
Rhinolophidae	Schneider's roundleaf bat	*Hipposideros speoris*	Japanese encephalitis virus
Rhinolophidae	Stripped roundleaf bat	*Hipposideros vittatus*	Hepacivovirus
Vespertilionidae	Savi's pipistrellle	*Hypsugo savii*	Astrovirus sp.
Vespertilionidae	Great evening bat	*Ia io*	Astrovirus sp.
Vespertilionidae	Great evening bat	*Ia io*	*Io io* picronavirus
Pteropodidae	Long-tongued fruit bat	*Macroglossus minimus*	Carey Island virus
Megadermatidae	Greater false vampire bat	*Megaderma lyra*	Astrovirus sp.
Pteropodidae	Peter's dwarf epauletted fruit bat	*Micropteropus pusillus*	Rift Valley fever virus
Vespertilionidae	Japanese long-fingered bat	*Miniopterus fuliginosus*	Japanese encephalitis virus
Vespertilionidae	Japanese long-fingered bat	*Miniopterus fuliginosus*	Yokose virus
Vespertilionidae	Western long-fingered bat	*Miniopterus magnate*	Astrovirus sp.
Vespertilionidae	Western long-fingered bat	*Miniopterus magnate*	Picrornavirus, group 2
Vespertilionidae	Small long-fingered bat	*Miniopterus pusillus*	Astrovirus sp.
Vespertilionidae	Small long-fingered bat	*Miniopterus pusillus*	Picornavirus, group 1
Vespertilionidae	Small long-fingered bat	*Miniopterus pusillus*	Picornavirus, group 2
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Astrovirus sp.
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Japanese encephalitis virus
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* picornavirus 1
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Mischivirus
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Picornavirus, group 1
Vespertilionidae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	Rift Valley fever virus
Molossidae	Black mastiff bat	*Molossus ater*	Dengue 2 virus

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Molossidae	Black mastiff bat	*Molossus ater*	Ilheus virus
Molossidae	Black mastiff bat	*Molossus ater*	Manzanilla virus
Molossidae	Black mastiff bat	*Molossus ater*	Mucambo virus
Molossidae	Black mastiff bat	*Molossus ater*	Rio Bravo virus
Molossidae	Black mastiff bat	*Molossus ater*	St. Louis encephalitis virus
Molossidae	Black mastiff bat	*Molossus ater*	Tamana bat virus
Molossidae	Black mastiff bat	*Molossus ater*	Yellow fever virus
Molossidae	None	*Molossus major*	St. Louis encephaltitis virus
Molossidae	None	*Molossus major*	Tamana bat virus
Molossidae	Pallas' mastiff bat	*Molossus molossus*	Dengue 2 virus
Molossidae	Pallas' mastiff bat	*Molossus molossus*	Ilheus virus
Molossidae	Pallas' mastiff bat	*Molossus molossus*	Influenza A virus H18N11
Molossidae	Pallas' mastiff bat	*Molossus molossus*	St. Louis encephalitis virus
Molossidae	None	*Molossus obscurus*	Catu virus
Molossidae	Angolan free-tailed bats	*Mops condylurus*	Bukalasa bat virus
Molossidae	Angolan free-tailed bats	*Mops condylurus*	Dakar bat virus
Molossidae	Angolan free-tailed bats	*Mops condylurus*	Entebbe salivary gland virus
Molossidae	Angolan free-tailed bats	*Mops condylurus*	Pegivirus
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	Astrovirus sp.
Vespertilionidae	Little tube-nosed bat	*Murina aurata*	Japanese encephalitis virus
Vespertilionidae	Great tube-nosed bat	*Murina leucogaster hilgendorfi*	Japanese encephalitis virus
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	Astrovirus
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	Hepesvirus
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	Issyk-Kul virus
Vespertilionidae	Large myotis	*Myotis chinensis*	Astrovirus sp.
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	Astrovirus sp.
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	Hepevirus
Vespertilionidae	Geoffroy's bat	*Myotis emarginatus*	Astrovirus sp.
Vespertilionidae	Geoffroy's bat	*Myotis emarginatus*	Hepesvirus
Vespertilionidae	Horsfield's bat	*Myotis horsfieldii*	Astrovirus sp.

Vespertilionidae	Little brown bat	*Myotis lucifugus*	Montana *Myotis* leukoencephalitis virus
Vespertilionidae	Little brown bat	*Myotis lucifugus*	St. Louis encephalitis virus
Vespertilionidae	Little brown bat	*Myotis lucifugus*	West Nile virus
Vespertilionidae	Big-footed myotis	*Myotis macrodactylus*	Japanese encephalitis virus
Vespertilionidae	Large mouse-eared bat	*Myotis myotis*	Astrovirus sp.
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Ahun nairovirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Astrovirus sp.
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Japanese encephalitis virus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Mammalian orthoreovirus
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Astrovirus sp.
Vespertilionidae	Black myotis	*Myotis nigricans*	Dengue virus
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Astrovirus sp.
Vespertilionidae	Northern long-eared bat	*Myotis septentrionalis*	West Nile virus
Vespertilionidae	Yuma myotis	*Myotis yumanensis*	Kern Canyon virus
Vespertilionidae	Myotis	*Myotis* sp.	Eastern encephalitis virus
Mystacinidae	Lesser shot-tailed bat	*Mystacina tuberculate*	New Zealand hepevirus
Natalidae	Mexican funnel-eared bat	*Natalus stramineus*	Dengue virus
Natalidae	Trinidadian funnel-eared bat	*Natalus tumidirostris*	Bimiti virus
Natalidae	Trinidadian funnel-eared bat	*Natalus tumidirostris*	Ilheus virus
Natalidae	Trinidadian funnel-eared bat	*Natalus tumidirostris*	St. Louis encephalitis virus
Vespertilionidae	Banana pipistrelle	*Neoromicia nanus*	Mouyassué virus
Noctilionidae	Great bulldog bat	*Noctilio leporinus*	Rio Bravo virus
Noctilionidae	Great bulldog bat	*Noctilio leporinus*	Venezuelan equine encephalitis virus
Vespertilionidae	Common noctule	*Nyctalus noctule*	Astrovirus sp.
Vespertilionidae	Common noctule	*Nyctalus noctule*	H3N2 Influenza A virus
Vespertilionidae	Common noctule	*Nyctalus noctule*	Issyk-Kul virus
Vespertilionidae	Common noctule	*Nyctalus noctule*	Mammalian orthoreovirus
Emballonuridae	Gambian slit-faced bat	*Nycteris gambiensis*	Fomédé virus
Emballonuridae	Gambian slit-faced bat	*Nycteris gambiensis*	Saboya virus

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Emballonuridae	Hairy slit-faced bat	*Nycteris hispida*	Magboi virus
Emballonuridae	Dwarf slit-faced bat	*Nycteris nana*	Fomédé virus
Vespertilionidae	Large-eared free-tailed bat	*Otomops martiensseni*	Hepaciviovirus
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	Influenza A virus H18N11
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	Tamana bat virus
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	Venezuelan equine encephalitis virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Bimiti virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Catu virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Dengue 2 virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Eastern equine encephalitis virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Guama virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Ilheus virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Influenza A virus H18N11
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Mucambo virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	St. Louis encephalitis virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Tamana bat virus
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	Yellow fever virus
Vespertilionidae	Japanese pipistrelle	*Pipistrellus abramus*	Astrovirus sp.
Vespertilionidae	Japanese pipistrelle	*Pipistrellus abramus*	Huangpi virus
Vespertilionidae	Japanese pipistrelle	*Pipistrellus abramus*	Japanese encephalitis virus
Vespertilionidae	Japanese pipistrelle	*Pipistrellus abramus*	Picornavirus sp., group 2
Vespertilionidae	Kuhl's pipistrelle	*Pipistrellus kuhlii*	Mammalian orthoreovirus
Vespertilionidae	Kuhl's pipistrelle	*Pipistrellus kuhlii*	Toscana virus
Vespertilionidae	Nathusius's pipistrelle	*Pipistrellus nathusii*	Mammalian orthoreovirus
Vespertilionidae	Common pipistrelle	*Pipestrellus pipistrellus*	Ahun nairovirus
Vespertilionidae	Common pipistrelle	*Pipestrellus pipistrellus*	Astrovirus sp.
Vespertilionidae	Common pipistrelle	*Pipestrellus pipistrellus*	Issyk-Kul virus
Vespertilionidae	Common pipistrelle	*Pipestrellus pipistrellus*	Mammalian orthoreovirus
Vespertilionidae	Common pipistrelle	*Pipestrellus pipistrellus*	Sokoluk virus

Vespertilionidae	Pipistrelles	*Pipestrellus* sp.	Bangui virus
Phyllostomidae	Recife broad-nosed bat	*Platyrrhinus helleri*	Tacribe virus
Phyllostomidae	Recife broad-nosed bat	*Platyrrhinus recifinus*	Influenza A virus H18N11
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	Astrovirus sp.
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	Japanese encephalitis virus
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	Mammalian orthoreovirus
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	Dengue 2 virus
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	Ilheus virus
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	St. Louis encephalitis virus
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	Yellow fever virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Bimiti virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Dengue 2 virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Ilheus virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Rio Bravo virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	St. Louis encephalitis virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Tamana bat virus
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	Yellow fever virus
Pteropodidae	Variable flying fox	*Pteropus hypomelanus*	Pulau virus
Pterpodidae	Gray-headed flying fox	*Pteropus poliocephalis*	Nelson Bay orthoreovirus
Pteropodidae	Little red flying fox	*Pteropus scapulatus*	Broome virus
Pteropodidae	Large flying fox	*Pteropus vampyrus*	*Pteropus orthoreovirus*
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Astrovirus sp.
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Longquan virus
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* picornavirus I
Rhinolophidae	Little Japanese horseshoe bat	*Rhinolophus comutus*	Japanese encephalitis virus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Astrovirus sp.
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Hantaan virus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Japanese encephalitis virus
Rhinolophidae	Eloquent horseshoe bat	*Rhinolophus hildebrandtii eloquens*	Mount Elgon bat

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Bat rotavirus A
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Mammalian orthoreovirus, reassortment
Rhinolophidae	Horseshoe bats	*Rhinolophus* sp.	Sindbis virus
Phyllostomidae	Dwarf little fruit bat	*Rhinophylla pumilio*	Influenza A virus H18N11
Rhinolophidae	Indian flying fox	*Pteropus giganteus*	Pegivirus
Rhinolophidae	Little Japanese horseshoe bat	*Rhinolophus comutus*	Oita virus
Rhinolophidae	Eloquent horseshoe bat	*Rhinolophus hildebrandtii eloquens*	Mount Elgon bat virus
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Astrovirus sp.
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Bat rotavirus 1
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Orthoreovirus, reassortment
Rhinolophidae	Formosan lesser horseshoe bat	*Rhinolophus monoceros*	Longquan virus
Rhinolophidae	Pearson's horseshoe bat	*Rhinolophus pearsonii*	Astrovirus sp.
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus*	*Mammalian orthoreovirus*, reassortment
Rhinolophidae	Rufous horseshoe bat	*Rhinolophus rouxi*	Astrovirus sp.
Rhinolophidae	Rufous horseshoe bat	*Rhinolophus rouxi*	Japanese encephalitis virus
Rhinolophidae	Rufous horseshoe bat	*Rhinolophus rouxi*	Kyasanur Forest disease virus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Longquan virus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Picornavirus sp., group 3
Emballonuridae	Proboscis Bat	*Rhynchonycteris naso*	Eastern equine encephalitis virus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Chikungunya virus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Kasokero
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	West Nile virus
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	Yogue
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Astrovirus sp.
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Chikungunya virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Influenza A virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Japanese encephalitis virus

Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Kyasanur Forest disease virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Malsoor virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	West Nile virus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Xi River virus
Pteropodidae	Rousettes	*Rousettus* sp.	Uganda S virus
Vespertilionidae	Lesser house bat	*Scotophilus* sp.	Bangui virus
Vespertilionidae	Lesser house bat	*Scotophilus* sp.	Chikungunya virus
Vespertilionidae	Lesser house bat	*Scotophilus* sp.	Dakar bat virus
Vespertilionidae	Lesser house bat	*Scotophilus* sp.	Hepacivirus
Vespertilionidae	Lesser Asiatic yellow house bat	*Scotophilus kuhlii*	Astrovirus sp.
Vespertilionidae	Lesser Asiatic yellow house bat	*Scotophilus kuhlii*	Keterah virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	Eastern equine encephalitis virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	H17N10 Influenza A virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	St. Louis encephalitis virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	Venezuelan equine encephalitis virus
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	Vesicular stomatitis virus
Phyllostomidae	Highland yellow-shouldered bat	*Sturnira ludovici*	Venezuelan equine encephalitis virus
Phyllostomidae	Yellow-shouldered bats	*Sturnira* sp.	Ilheus sp.
Phyllostomidae	Yellow-shouldered bats	*Sturnira* sp.	St. Louis encephalitis virus
Pteropodidae	Long-tongued fruit bat	*Sycnycteris crassa*	Japanaut virus
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	Rio Bravo virus
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	St. Louis encephalitis virus
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	Tamana bat virus
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	West Nile virus
Molossidae	Angolan free-tailed bat	*Tadarida condylura*	Bukalasa virus
Molossidae	Angolan free-tailed bat	*Tadarida condylura*	Dakar bat virus.
Molossidae	Ferruginous Glider	*Tadarida limbata*	Entebbe bat salivary gland virus
Molossidae	Little free-tailed bat	*Tadarida pumila*	Bukalasa bat virus
Molossidae	Free-tailed bats	*Tadarida* sp.	Bangui virus

(*Continued*)

TABLE 6.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Molossidae	Free-tailed bats	*Tadarida* sp.	Gossas virus
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	Astrovirus sp.
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	Japanese encephalitis virus
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	Laibin virus
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	Yokose virus
Emballonuridae	Egyptian tomb bat	*Taphozous perforatus*	Dakar bat virus
Emballonuridae	Teobald's tomb bat	*Taphozous theobaldi*	Kaeng Khoi virus
Phyllostomidae	Greater round-eared bat	*Tonatia bidens*	Tacaribe virus
Vespertilionidae	Greater bamboo bat	*Tylonycteris robustula*	Astrovirus sp.
Vespertilionidae	Greater bamboo bat	*Tylonycteris robustula*	Tacaribe virus
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	Venezuelan equine encephalitis virus
Phyllostomidae	Great stripe-faced bat	*Vamipyrodes caraccioli*	Hepevirus
Phyllostomidae	Great stripe-faced bat	*Vamipyrodes caraccioli*	Vesicular stomatitis virus
Phyllostomidae	Bidentate yellow-eared bat	*Vampyressa bidens*	Influenza virus A H18N11
Phyllostomidae	Heller's broad-nosed bat	*Vampyrops helleri*	Eastern equine encephalitis virus
Phyllostomidae	Heller's broad-nosed bat	*Vampyrops helleri*	Ilheus virus
Phyllostomidae	Heller's broad-nosed bat	*Vampyrops helleri*	Yellow fever virus
Vespertilionidae	Parti-colored bat	*Vespertilio murinus*	Astrovirus sp.
Vespertilionidae	Asian parti-colored bat	*Vespertilio serotinus*	Issyk-Kul virus
Vespertilionidae	Asian parti-colored bat	*Vespertilio superans*	Japanese encephalitis virus

(discussed below) on Tioman Island was 13% ($n = 109$) (Chua *et al*. 2007). Since RNA from these two viruses is similar, they may induce cross-reactive antibodies. A third fusogenic bat orthoreovirus, Xi River virus, was more recently isolated from pooled lung tissue of *Rousettus leschenaultia* in China (Du *et al*. 2010). Another member of this group of orthoreoviruses, Indonesia/2010, was also isolated from a salivary swab from a healthy large flying fox (*Pteropus vampyrus*) from Indonesia that had been imported into Italy (Lorusso *et al*. 2015).

Melaka virus, a similar pteropine fusogenic orthoreovirus, was discovered in a man with acute respiratory disease (Chua *et al*. 2007). Two of the man's children also developed a high fever approximately a week later, suggesting that Melaka virus may be capable of human-to-human transmission. The man's pregnant wife did not become ill and delivered a healthy child soon afterwards. Of note, a bat had entered and flown about the family's house about 1 week before the beginning of the father's clinical symptoms. The dsRNA sequence of the Melaka virus is closely related to those of Nelson Bay and Pulau viruses (Chua *et al*. 2007).

Kampar virus is another Melaka-like reovirus from humans that causes cytopathic effect in kidney cell lines *in vitro*. It was isolated from a throat swab of a Malaysian having high fever, acute respiratory disease, and vomiting (Chua *et al*. 2008). The virus was transmitted to and caused respiratory disease in at least one other person. The wife and physician of the index patient were seropositive for Kampar virus. The genomes of Kampar and Melaka viruses are closely related and both are neutralized by serum against the other. It has been suggested that these viruses possibly originated in bats since the house of the patient with Kampar virus is surrounded by fruit trees frequented by fruit bats. Even though partially eaten fruit was found near the index patient's house, no bats were known to have entered the house and no dead bats were found in the house's grounds (Chua *et al*. 2008). No bats have been reported to be infected with these viruses. Additionally, no mention was made of the affected people being bitten by bats or having consumed the partially eaten fruit. There is no good evidence, therefore, to support the contention of a bat origin for either virus.

In Hong Kong, another orthoreovirus, reovirus strain HK23629/07, was isolated from a patient with high fever, an acute respiratory infection, and diarrhea. The patient had recently travelled to Bali, Indonesia. The HK virus has high deduced amino acid homology (88–98%) with Melaka virus, except for in the virus-cell attachment protein, in which the homology was 67%. This suggests that this virus is similar to the bat Melaka virus and is a member of the Nelson Bay virus group (Cheng *et al*. 2009). However, given the large differences in the viruses' attachment proteins, critical to host cell tropism, it is questionable whether HK virus is able to enter cells from a different host genus. Another similar orthoreovirus was isolated from a throat swab from a Japanese traveler to Bali, who developed a high fever, joint pain, sore throat, and cough. The viral isolate was named "Miyazaki" and is most closely related to the human isolate HK23629/07 (Yamamanaka *et al*. 2014). No connection to bats was found.

A phylogenetic tree based on the most variable protein suggests that Pulua virus is more closely related to the human isolates (Melaka, Kampar, HK, and Miyazaki viruses) than to Nelson Bay virus. The latter has nucleotide sequence identity ranging from 56 to 88% for different segments of the dsRNA genome (Pritchard *et al*. 2006; Chua *et al*. 2008).

It has been proposed that all of the above orthoreoviruses be placed into one genus designated *Pteropine orthoreovirus* (Chua *et al*. 2011).

A seventh member of this proposed genus is the fusogenic Sikamat virus, isolated from a throat swab of a male patient in Malaysia who had a high fever of sudden onset, severe sore throat and headache, prostrating myalgia, and moderate epigastric pain, but no respiratory symptoms (Chua *et al*. 2011). He spent his weekends in a house within an orchard. At night, fruit bats flew around in the orchard and occasionally entered the house, but without roosting there. The patient did not directly handle or kill any bats nor did he consume partially eaten fruit or fruit dropped on the ground. The patient's wife and one son seroconverted but were asymptomatic. The son did not spend the nights in the orchard house (Chua *et al*. 2011).

Interestingly, all three of the outbreaks among humans in Malaysia were associated with multiple human infections, with some infected people being asymptomatic. Epidemiological tracing indicates that these outbreaks were more likely to have resulted from human-to-human transmission than to independent spillover from bat orthoreoviruses (Chua *et al*. 2011), even in light of the outbreaks' tenuous linkages to bats. It would be useful to determine whether other animals in the area, particularly birds with their fusogenic reoviruses, harbor similar orthoreoviruses.

6.2.4 Mammalian orthoreoviruses

In contrast to the members of the *Pteropine orthoreovirus* group described above, mammalian orthoreoviruses (MRVs) are not fusogenic and generally do not kill host cells. The species contains four prototype strains: type 1 Lang, type 2 Jones, type 3 Dearing, and type 4 Ndelle. The strains do not cross-react serologically (Kohl *et al*. 2012). This group of reoviruses includes human pathogens responsible for severe diseases, including acute respiratory infections and central nervous system disorders, as well as hemorrhagic enteritis in dogs (reviewed by Steyer *et al*. 2013).

A wide range of cell lines are infected by MRVs, but they have a preference for transformed cells. A 2013 study found that MRV type 3 Dearing can replicate and produce infectious progeny in a transformed bat lung epithelial cell line derived from *Tadarida brasiliensis*. The infection is transient, decreasing rapidly to low titer. Importantly, the virus did not produce cytopathic effect or kill the cells (Sandekian *et al*. 2013). Afterwards, the cells are resistant to reinfection and produce an antiviral factor that is believed to be interferon since the cells are also protected against infection by the unrelated murine encephalomyocarditis virus. MRV are relatively stable and may be found in environmental samples.

In Slovenia, a MRV was discovered in a stool sample of a hospitalized 17-month-old child with acute gastritis. The identification of the virus as a reovirus utilized genomic sequencing in addition to visual examination. The child recovered 8 days after the onset of diarrhea. The new MRV, designated Slovenian SI-MRV01, had the closest degree of similarity to the German bat MRVs described below. The nucleotide and amino acid identities for all of the genetic segments were 93.8–99.0% and 98.4–99.7%, respectively (Steyer *et al*. 2013). The source of the infection is not known, however, the child's residence was in an area from which no bats had been observed. The only animal having had close contact with the child was the child's grandfather's dog. The bat MRVs were identified in insectivorous bats, ruling out the possibility of infection by consumption

of partially eaten fruit. The child also ate non-food items, however, so infection via environmental means, even though unlikely, cannot be ruled out.

In addition to the fusogenic *Pteropine orthoreovirus* from Southeast Asian and Australian fruit bats, three MRVs were recently found in European bats, greatly expanding the geographic range of known bat-borne orthoreovirus. Moreover, these MRVs infect insectivorous bats (*Plecotus auritus, Myotis mystacinus, Pipistrellus pipistrellus, Pipistrellus nathusii, P. kuhlii*, and *N. nocule*) (Kohl *et al*. 2012). Approximately 6.7% of tested vespertilionid bats ($n = 120$) were infected with one of three novel MRV isolates. These viruses have particular tropism for the intestine, in keeping with the diarrheal symptoms described in dogs and the child above, but also may be found in other tissues. Pathology in infected bats included hemorrhagic enteritis (intestine), nonsuppurative interstitial pneumonia (lungs), follicular hyperplasia (spleen), and glomerulopathy (kidneys). The strain T3/Bat/Germany/342/08 was found to have the closest phylogenetic relationship to MRV strain T3D/04, isolated previously from a dog (Kohl *et al*. 2012). The placement of the bat isolates in the MRV group is supported by its terminal 5′ RNA segment sequences, which are conserved among each species, but differ from the others. They are used as phylogenetic markers for virus type differentiation. All terminal sequences for the German bat strains are similar to those of MRV. T3/Bat/Germany/342/08 strain also is nonfusogenic and its S1 genomic segment is bicistoronic rather than the tricistronic S1 that is characteristic of *Pteropine orthoreovirus* bats (Kohl *et al*. 2012).

Another important property of MRVs is their ability to reassort, allowing their spread to new, immunologically naïve hosts. A type 2 MRV, RpMRV-YN2012, was isolated from pooled urine samples from the least horseshoe bat (*Rhinolophus pusillus*) in China. Four anal swabs (16%; $n = 25$) from these bats also contained virus. Genomic analysis of the urine samples revealed that one of the gene segments (S1) was most closely related to a pig type 1 MRV (nucleotide and amino acid identities of 93.9–96.2 and 96.8–97.9%, respectively), while three other segments (S2–S4) were highly similar to the bat type 2 MRV strain 342/08 from Germany (nucleotide and amino acid identities of 94.9–98.1 and 98.3–99.2%, respectively) (L. Wang *et al*. 2015). A separate study isolated a novel reassortment type 1 MRV, designated BatMRV1-IT2011, from fecal samples from apparently healthy lesser horseshoe bats (*R. hipposideros*) in Italy (Lelli *et al*. 2015). This MRV appears to be a reassortment strain which contains an S1 genome segment similar to those of bovine MRV T1 strains, while the other segments are more similar to other MRVs, especially those causing enteric, respiratory, or encephalitic disorders in humans and other animals. This bat MRV, however, has not been linked to human infection.

Thalmann *et al*. (2010) isolated another fusogenic orthoreovirus from pooled lung, liver, spleen, and kidney tissues from little red flying fox bats (*Pteropus scapulatus*) in Australia. This virus, designated as Broome virus, is unique in being fusogenic but having a monocistronic S1 genomic segment. It is not cross-reactive with antibodies to the Nelson Bay virus group and it shares only 13–50% amino acid identity with other orthoreoviruses. It also has unique terminal 5′ RNA segment sequences on the plus strand as well as a unique fusion protein. For these reasons, it was recommended to be placed into a new, separate species group of *Orthoreoviridae* (Thalmann *et al*. 2010). The bat from which Broome virus was isolated displayed aggression, hind-limb paresis, and generalized weakness. Since its brain, however, was

infected by Australian bat lyssavirus, the noted symptoms are not likely to be related to the Broome virus infection.

6.3 BALTIMORE CLASS IV VIRUSES

6.3.1 Astroviruses

Astroviruses are small, spherical ssRNA (+) viruses whose genome contains three overlapping open reading frames and is polyadenylated at the 3′ terminus. They lack an envelope, but possess numerous short projections from their surface, giving them a star-shaped appearance. They infect many species of vertebrates, including humans. Astroviruses are classified into the *Mamastrovirus* and *Avastrovirus* genera found in mammals and birds, respectively. Mamastroviruses are divided into seven monophyletic groups, with group 1 primarily composed of viruses from humans. Astroviruses from bats have a high degree of genetic diversity, with five of the six remaining groups found only in bats. Group 4 also contains astroviruses from sheep and mink (Zhu *et al*. 2009).

Infection is typically associated with a self-limiting gastroenteritis, accounting for 2–9% of all acute nonbacterial gastroenteritis cases in children worldwide. Disease may be severe in immunocompromised adults or the elderly. The viruses infect enterocytes and transmission is via the oral–fecal route. Given the wide range of vertebrate hosts, zoonotic spread to humans is possible from many mammals.

In Hong Kong, astroviral RNA with a high degree of genetic diversity was reported in 46% of anal swabs and 9% of oral swabs from healthy bats ($n = 262$). Seven bat species were found to harbor astrovirus RNA: 51% of western long-fingered bats (*Miniopterus magnater*) ($n = 67$), 43% of small long-fingered bats (*Miniopterus pusillus*) ($n = 32$), 100% of Schreiber's long-fingered bats (*Miniopterus schreibersii*) ($n = 3$), 33% of the large myotis (*Myotis chinensis*) ($n = 9$), 83% of Rickett's big-footed myotis (*Myotis ricketii*) ($n = 12$), 33% of Japanese pipistrelles (*Pipistrellus abramus*) ($n = 3$), and 25% of rufous horseshoe bats (*Rhinolophus rouxi*) ($n = 8$) (Chu *et al*. 2008). A large number of the bats were co-infected with bat coronaviruses. Since these two viral groups have been known to recombine, this is a matter of zoonotic concern.

In order to better comprehend the geographic range and diversity of astroviruses in bats in mainland China, 500 anal swabs were collected from 20 bat species from 51 sites throughout the country. Astrovirus RNA was present in 44.8% of tested bat samples from 32 of the sites (Zhu *et al*. 2009). Viral prevalence was highest in bearded tomb bats (93% of *Taphozous melanopogon* samples) and 63.2% of *M. schreibersii*. All viruses belonged to the *Mamastrovirus* genus and, as is the case in other studies, all positive bats were insectivorous or carnivorous and, thus, these findings may represent passage through the digestive tract rather than true infection.

A more recent study of healthy Chinese bats found astrovirus RNA in 10 bat species ($n = 19$) (Hu *et al*. 2014). The overall prevalence rate was 7.6% (range = 2.4–75%). The highest prevalence rates were present in Western bent-wing bats (*M. magnater*) (75%; $n = 4$), intermediate roundleaf bats (*Hipposideros larvatus*) (17.7%; $n = 79$), and lesser Asiatic yellow house bats (*Scotophilus kuhlii*) (11.5%; $n = 130$). Astroviruses were

found in three bat species, the least horseshoe bat (*Rhinolophus pusilus*) (10%; $n=20$), the intermediate horseshoe bat (*Rhinolophus affinis*) (2.4%; $n=84$), and Horsfield's bat (*Myotis horsfieldii*) (11.8%; $n=17$). Astroviruses were also detected in great roundleaf bats (*Hipposideros armiger*) (14.3%; $n=7$), *M. schreibersii* (6.5%; $n=93$), Rickett's big-footed bats (*Myotis ricketti*) (5.3%; $n=19$), and greater bamboo bats (*Tylonycteris robustula*) (11.1%; $n=9$). The majority of the tested bat populations exhibited host restriction: group 1 reported exclusively in Hipposideridae and Rhinolophidae; groups 2 and 4, in the *Myotis* and *Miniopterus* genera (family Vespertilionidae), respectively; and group 3, only detected in *S. kuhlii* (Hu *et al*. 2014). Nevertheless, a high degree of astrovirus diversity was found within bat species, however, with several virus species present in the same individual bat. Xiao *et al*. (2011) had previously detected astrovirus RNA in healthy insectivorous *M. schreibersii* (11.8%; $n=187$) and *S. kuhlii* (15.8%; $n=38$) and also in the frugivorous Leschenault's rousette (*R. leschenaultia*) (1.7%; $n=59$). Insectivorous or carnivorous bats appear to be much more likely to harbor astroviruses than do frugivorous bats.

In Europe, astrovirus prevalence in bats was accessed by studies in Hungary and the Czech Republic. In a study of fecal samples from 60 bats, 42.8% of tested *Myotis daubentonii* were positive for astrovirus RNA ($n=7$), 9.1% of *P. auritus* ($n=11$), and 4.5% of *Myotis bechsteinii* ($n=22$) (Kemenesi *et al*. 2014). Phylogenetic analysis indicated that the Hungarian astrovirus isolates clustered together with those from China and Europe and were separate from astroviruses of other mammals. A separate study of *M. myotis* in Europe discovered six distinct mamastroviruses with 65.0–86.0% amino acid identities with other bat-associated astroviruses from *M. chinensis* and *M. ricketti* bats from China (Drexler *et al*. 2011).

Astrovirus RNA was detected in nine bat species in the Czech Republic: *Eptesicus serotinus, Hypsugo savii, Myotis emarginatus, M. mystacinus, Nyctalus noctula, P. nathusii* or *Pipestrellus pygmaeus, P. pipistrellus, Vespertilio murinus*, and *R. hipposideros*. An astrovirus strain from *R. hipposideros* clustered phylogenetically with astrovirus strains from Chinese Rhinolophidae and Hipposideridae bats. The other Czech astrovirus RNA sequences in this study, as well as a Hungarian sequence, formed a separate monophyletic lineage (Dufkova *et al*. 2015). A 2016 study examined the prevalence of astroviruses in urine and oral and fecal swabs from four bat species in Germany ($n>950$): Natterer's bats (*Myotis nattereri*), *M. bechsteinii*, the Daubenton's myotis (*M. daubentonii*), and brown long-eared bats (*P. auritus*) (Fischer *et al*. 2016). Overall prevalence of astroviruses was 25.8%, reaching as high as 63.8% in *M. daubentonii* ($n=47$). Sixteen different RNA sequences were found in *M. daubentonii*, indicating a high degree of diversity. Genomic similarities of astroviruses within a bat species at different sites, however, were greater than that among different species at the same site. Interestingly, of the 16 astrovirus sequences in *M. daubentonii* in Germany, 14 clustered with astroviruses from *M. daubentonii* in Hungary, despite the fact that these bats do not generally migrate more than 50 km (Fischer *et al*. 2016).

Interestingly, a strong and specific amplification of RNA viruses, including astroviruses, occurs during colony formation and may be linked to the establishment of a large and dense contiguous population of susceptible adult bats. Amplification may also occur after parturition during times in which new virus lineages become predominant in the bat population (Drexler *et al*. 2011).

6.3.2 Flaviviruses

Flaviviruses have a ssRNA (+) genome. Some of these viruses cause serious to fatal diseases in humans. The highly pathogenic yellow fever virus was the first virus found to be a filterable causative agent of a severe human disease. Dengue virus types 1–4 often are associated with fever and severe bone ache in humans, however infection may lead to the fatal diseases, dengue hemorrhagic fever and dengue shock syndrome that are largely linked to antibody-dependent enhancement that may occur following infection with a second dengue serotype. Other flaviviruses, including Japanese encephalitis virus (JEV), St. Louis encephalitis (SLE) virus, West Nile virus (WNV), Western equine encephalitis virus, and tick-borne encephalitis virus cause severe encephalitis in humans. Flaviviruses are typically divided into groups known to be transmitted by mosquitoes and ticks or flaviviruses with no known vector.

6.3.2.1 Dengue virus Dengue has been considered for many years by the World Health Organization as a pandemic that is increasing in scope as its four serotypes spread. It is deemed to be the most important mosquito-borne viral disease for humans and is endemic in over 100 countries. Dengue serotypes infect 100 million people annually and are linked to over 25 000 deaths, primarily in children under 5 years of age. People who have been infected by more than one serotype are at much greater risk of developing the more severe forms of the disease. Different serotypes of dengue have been reported in bats in many parts of the world. Dengue has been detected in the frugivorous *Pteropus* species bats in Australia (O'Connor *et al*. 1955).

In Central and South America, neutralizing antibodies against dengue-1 (DENV-1) and dengue-2 (DENV-2) were present in 22.6% ($n=53$) and against dengue-3 (DENV-3) in 30.0% ($n=10$) of bats from Costa Rica and Ecuador, respectively (Platt *et al*. 2000). All four dengue serotypes are present in Mexican bats. A 2008 study in that country examined 162 bat heart tissues from five families: Emballonuridae, Mormoopidae, Phyllostomidae, Natalidae, and Vespertilionidae, encompassing 12 genera and 19 species, of which eight were frugivorous, seven insectivorous, three nectivorous, and one hematophagous. The following bat species were seropositive: three insectivorous bats – 33% of *Myotis nigricans* ($n=12$), 15.8% of *Pteronotus parnellii* ($n=19$), and 25% of *Natalus stramineus* ($n=4$) as well as in one frugivorous bat – 2.9% of *Artibeus jamaicensis* ($n=35$). DENV-2 RNA was additionally detected in 50% of the frugivorous *Carollia brevicauda* ($n=2$) (Aguilar-Setién *et al*. 2008). A more recent study conducted in southeastern Mexico examined whether human alteration of the ecosystem altered DENV-2 prevalence in bats (Sotomayor-Bonilla *et al*. 2014). Spleen samples from 146 bats, belonging to 16 bat species, were tested for the presence of viral RNA for the four dengue virus serotypes. DENV-2 RNA was detected in 4.1% of the bats: two *Glossophaga soricina*, three *Artibeus lituratus*, and one *A. jamaicensis*. It is noteworthy that anthropogenic disturbance did not appear to alter the prevalence of dengue infection of bats (Sotomayor-Bonilla *et al*. 2014).

In order to examine whether *A. jamaicensis* bats were able to sustain infection with dengue and thus indirectly transmit it to other vertebrates, bats were inoculated with different dengue virus serotypes via different routes (Cabrera-Romo *et al*. 2014). One group of animals was inoculated subcutaneously and another intraperitoneally with DENV-4, while another group was inoculated intraperitoneally with DENV-1. The final group of bats was bitten by *Aedes aegypti* mosquitoes harboring DENV-1 or DENV-4.

No viral RNA was detected by PCR analysis of plasma or spleen tissue on day 1 up to 9–17 days post-infection. Additionally, no specific anti-DENV IgG was found in the bats' plasma and no clinical and behavioral changes were evident in the inoculated bats. Another similar study of 23 *A. intermedius* following intraperitoneal inoculation with DENV-2 found structural alterations in the spleen and bleeding in the liver and intestines, but failed to detect viral RNA in these tissues. Viral RNA was detected by semi-nested RT-PCR in 39% of the bats, however only 8% of bats seroconverted (Perea-Martínez *et al*. 2013). This is in keeping with several previous reports which also failed to detect dengue replication following intraperitoneal inoculation of 27 *A. intermedius* bats with DENV-2 or following experimental inoculation of North American bats with the closely related WNV or Australian black flying foxes with JEV (Davis *et al*. 2005; van den Hurk *et al*. 2009). Taken together, these results call into question whether *A. jamaicensis* or *A. intermedius* bats are able to sustain dengue virus replication or serve as dengue viral reservoirs.

6.3.2.2 Venezuelan encephalitis virus Venezuelan encephalitis (VE) virus is a mosquito-borne virus whose primary hosts are horses for the epizootic viral strains, which develop neurological disease. The enzootic strains use cotton rats as their primary host. Humans may also be infected by either group of viral strains via the bite of infected mosquitoes and typically experience flu-like disease, however, in immunocompromised people, including the young and elderly, the disease is severe and may lead to death. A survey of bats exposed to VE virus was conducted in the Pacific lowlands of Guatemala during 1978. The virus was isolated from blood of an *Uroderma biobatum* bat and VE virus-specific antibodies were found in seven bat species (Seymour *et al*. 1978a). Antibodies were also detected at a higher frequency in other mammals, including adolescent and adult humans, dogs, rodents, and opossums. The bat species with VE-specific antibodies were as follows: *A. jamaicensis, A. lituratus, Artibeus phaeotis, Desmodus rotundus, Glossophaga commissarisi,* and *M. nigricans*. The prevalence rate for seropositive bat species ranged from 3 to 12% (Seymour *et al*. 1978a).

Experimental inoculation of *A. jamaicensis, A. lituratus*, and *Phyllostomus discolor* with both enzootic and epizootic strains of VE produced viremia in 92.5% of the bats, without apparent illness. Viremia was also detected in experimentally infected *Caroliia subrufa* and *Sturnira lilium* bats, the majority of which died due to handling and the bleeding procedures. Maximal viremia in the former three bat species averaged 6.9, 6.6, and 4.6 logs, respectively, 4 days post-infection. These are sufficient viral levels to infect a vector, the *Culex fatigans* mosquito. Virus was present in very high titers in the oropharyngeal cavities of 56% of the experimentally infected bats, most frequently in *A. jamaicensis*. In contrast, virus was detected in only 1.6% of the urine samples ($n = 123$) and 2.3% of fecal samples ($n = 86$) (Seymour & Dickerman 1978). Transmission between bats did not occur by direct contact or aerosols, as it does in cotton rats, horses, and dogs. *Artibeus* species generally developed and maintained detectable levels of both hemagglutination-inhibition and neutralizing antibodies for the entire testing period (up to 506 days). The detectable antibody response of *P. discolor* was slower and of lower magnitude and shorter duration than that of *Artibeus* species, although individual *P. discolor* bats which lost detectable levels of both antibody types were still able to resist viral challenge. Secondary infection with VE failed to produce viremia (Seymour *et al*. 1978b).

6.3.2.3 Japanese encephalitis virus JEV has a fatality rate of 25–50% in humans and approximately one half of the survivors develop persistent neurological sequelae. Five genotypes (GI–GV) are recognized based on the sequence of the envelope gene. Predominant genotypes of JEV isolates demonstrate geographical and temporal differences. GIII is found throughout Southeast Asia and Oceania. It has circulated in China since 1949, but is now being replaced by GI (reviewed by Liu *et al*. 2013). While this virus infects many vertebrates, only pigs and waterfowl are implicated as the viral reservoirs. A study conducted from 2007 to 2009 examined the diversity of JEV isolates in China. Previous studies had found three viral isolates in *R. leschenaultia* and one isolate in *Murina aurata* bats, all of which led to neuromuscular disease when inoculated into suckling mice (Wang *et al*. 2009). These four isolates plus four newer isolates (all from healthy bats) were compared with each other and found to have very little genetic diversity, having full-sequence nucleotide and amino acid identities of 99.4–99.9% and all belonging to GIII (Liu *et al*. 2013). Bat isolates appear to evolve more slowly than isolates from humans, pigs, and mosquitoes. A separate study determined the prevalence of antibodies against JEV in bats of southern China. Of 336 serum samples, 12.8% were positive by ELISA and approximately 25% contained neutralizing antibodies (Cui *et al*. 2008). Interestingly, no viral RNA was detected in the brain or liver. Antibodies against the virus have also been detected in *H. armiger, Hipposideros bicolor, Hipposideros cineraceus, Hipposideros pomona, Hipposideros speoris, M. schreibersii, Myotis macrodactylus, Rhinolophus comutus, Rhinolophus ferrumequinum, R. rouxi*, and *Vespertilio superans* (Miura *et al*. 1970; Banerjee *et al*. 1988; Calisher *et al*. 2006).

In order to determine the identity of bat hosts of Japanese B encephalitis (JBE) virus in Japan, 1934 bats from 10 species were examined during 1963–1965. Virus was isolated from blood and brown fat of bats from several locations and 8% of the bats produced virus-specific neutralizing antibodies. In contrast, no virus was recovered from bats in the northernmost part of Japan, a region where few human cases occur, and only 3% of the bats had neutralizing antibodies (Miura *et al*. 1970; Sulkin *et al*. 1970). Bat populations were sampled in the spring soon after emerging from hibernation, in the summer months, in the fall prior to hibernation, and during their winter hibernation periods in order to determine if the virus could overwinter in bat populations. Interestingly, equal frequency of isolation and neutralizing antibodies were found during all four seasons of the year, despite the prevalence of human infections during the summer months (Sulkin *et al*. 1970). Other studies found no virus in the mosquito vectors or the other known natural hosts (birds and swine) from April to late June. From that time until late July, the virus was detectable in mosquitoes, but only appeared in birds and pigs in late July or early August and by late September, no virus was found in mosquitoes, birds, or pigs, supporting the hypothesis that bats or other unknown species of mammals may be one of the reservoirs involved in maintaining the virus population throughout the year. The following bat species were found in this study to be naturally infected with the JBE virus: *M. schreibersi fuliginosa, Rhinolophus cornutus cornutus, M. macrodactylus*, and *Vespertino supertins*. Interestingly, virus was only found in one of 112 gravid females examined. Seven percent of weanling bats also produced neutralizing antibodies (Miura *et al*. 1970; Sulkin *et al*. 1970). Several bat species in which viremia was not detected were found to have neutralizing antibodies: *R. ferrumequinum nippon* (27%; $n=79$), *P. abramus* (3%; $n=31$), *M. mystacinus* (16%; $n=25$), *P. auritus sacrimontis* (5%; $n=22$), and *Murina leucogastcar hilgendorfi* (50%; $n=2$) (Miura *et al*. 1970).

Plasma samples from 1459 bats collected in Japan during 1963–1965 were assayed for serologic evidence of infection with JBE virus by an *in vitro* plaque-reduction method. Neutralizing antibodies were demonstrated in 8% of the specimens of plasma from bats netted in Honshu and Kyushu, Japan, whereas only 3% of the specimens from bats netted in Hokkaido, the northernmost part of Japan, were positive.

Japanese encephalitis is an emerging disease in Australia. Since bats are reservoirs for lyssaviruses, a study was conducted in order to determine if the frugivorous flying foxes in Australia might serve as reservoirs for this virus as well (van den Hurk *et al*. 2009). *Pteropus alecto* bats were exposed to JEV by inoculation or by exposure to infected *Culex annulirostris* mosquitoes, a species known to feed on flying foxes. All bats remained asymptomatic. Virus-specific IgG antibodies developed in 60% ($n = 10$) of the animals exposed to infected mosquitoes and in all five inoculated bats. Low levels of viral RNA were detected in one of these five animals and the viremic bat, in turn, infected recipient mosquitoes. No viremia was seen in any of the flying foxes exposed via infected mosquito bites, nevertheless, two of the bats infected recipient mosquitoes. It is surprising that bats lacking detectable viremia were able to infect mosquitoes, but this phenomenon could be due to viral replication in the bats' skin or dendritic cells at the site of inoculation, without entering the bloodstream. Indeed, the authors observed some recipient mosquitoes feeding at the same site as the donor mosquitoes and may have ingested virus from cells and tissues damaged by the mosquito mouthparts (van den Hurk *et al*. 2009). These findings shed new light and pose new problems for studies of other possible viral reservoir hosts.

6.3.2.4 West Nile and St. Louis encephalitis viruses

Culex mosquitoes are the primary vectors for WNV while *Aedes* species are the vectors for SLE and dengue viruses. Wild birds, especially crows and jays, are the principal hosts, although other vertebrates may also be infected, including humans and horses. In the US, serum samples ($n = 97$) were tested for the presence of antibodies to WNV in big brown bats (*Eptesicus fuscus*). The prevalence of infection was 1–2% in several northern states (Bunde *et al*. 2006). Two of 150 dead bats in New York State (one *Myotis lucifugus* and one *E. fuscus*) were seropositive in 2000, not long after the arrival of WNV to the Western Hemisphere (Marfin *et al*. 2001). A northern long-eared bat (*Myotis septentrionalis*) also produced anti-WNV antibodies in 2002 (Pilipski *et al*. 2004). Antibodies to SLE virus were found in 9% of big and little brown bats in the northern US as well ($n = 390$) (Herbold *et al*. 1983). After subcutaneous inoculation with WNV, 29.2% ($n = 24$) of *E. fuscus* bats were viremic between days 2 and 6 post-inoculation (titers = 10–180 plaque forming units/ml), but displayed no clinical symptoms. Virus was not found in their oral swabs or tissue samples. In the same study, however, none of the inoculated *T. brasiliensis* seroconverted, but the report found that 1.3% of *T. brasiliensis* sera from Louisiana in an area in which the virus is endemic ($n = 149$) did have neutralizing antibody to WNV (titers = 20 and 40; 1:10 dilution) (Davis *et al*. 2005). It should be noted that the antibodies used in this study were able to cross-react with other flaviviruses, making it difficult to know the exact identity of virus that was present when using antibody-dependent assays. The study authors concluded that all of their data together suggest that *E. fuscus* and *T. brasiliensis* bats, two of the most common bat species in the US, are unlikely to serve as amplifying hosts for WNV (Davis *et al*. 2005).

Two epidemics of SLE occurred in southern Texas in 1964 and 1966. From the blood or spleen of 1649 Mexican free-tailed bats (*T. brasiliensis*), 26 strains of SLE virus were isolated and 20% of the bats had virus-specific neutralizing antibodies ($n=663$). Virus was isolated from bats in almost every month, including periods of time between outbreaks in which SLE virus could not be detected in mosquitoes, birds, or humans (Allen *et al*. 1970). These data suggest that *T. brasiliensis* may serve as a persistent reservoir host in which SLE virus resides between outbreaks in humans.

In an effort to determine species and prevalence of flaviviruses in the Yucatan peninsula of Mexico, serum was collected from 140 bats and assayed by plaque reduction neutralization test and PCR for the presence of WNV, SLE virus, and DENV 1– 4. Flavivirus-specific antibodies were detected in 19% of the bats ($n=140$). The prevalence of seropositivity were as follows: 33% of Pallas's long-tongued bats (*G. soricina*), 24% of the Jamaican fruit bats (*A. jamaicensis*), and 9% of the great fruit-eating bats (*A. lituratus*). The antibody titers were higher for DENV-2 or DENV-4 than the other tested flaviviruses, however no titers were greater than 80. Since all of the titers were low, the bats may have been infected with a different flavivirus (Machain-Williams *et al*. 2013).

6.3.2.5 Entebbe bat virus group Entebbe bat virus is closely related to yellow fever virus of humans. It was isolated from the salivary glands of a little free-tailed bat (*Chaerephon pumilus*) in Uganda in 1957 and then not again until 2011 near the original site. In the latter study, infectious virus was isolated from the spleen and lung but not the heart, liver, or kidney (Kading *et al*. 2015). Four infected laboratory workers developed mild to severe illnesses. The Entebbe bat virus was placed in a sister clade to the yellow fever virus clade and is most closely related to Sokoluk virus, recovered from *P. pipistrellus* bats and soft ticks in Kyrgyzstan (L'vov *et al*. 1973). Yokose virus was isolated from long-fingered bats (*Miniopterus fuliginosus*) in Japan in 1971 and later, Yokose-specific antibodies were detected in 2.7% of insectivorous bats from the Philippines ($n=36$) and 91% of samples from Malaysia ($n=26$) (Watanabe *et al*. 2010). Yokose virus is more closely related to Entebbe bat virus, Sokuluk virus, and Sepik virus than to the yellow fever virus (Tajima *et al*. 2005).

6.3.2.6 Tamana bat virus In a study of 384 bats in Trinidad, 15.3% of the bats were seropositive for Tamana bat virus. The majority of infected bats were common vampire bats (*D. rotundus*; 45.7%) and Pallas's mastiff bat (*Molossus major*; 30.4%) (Thompson *et al*. 2015). Since *D. rotundus* is hematophagous, there is a potential risk to humans and livestock. Other flaviviruses detected in bats during the study include VE virus (2.9%), SLE virus (1.8%), and Rio Bravo virus (1.0%). None of the tested bats were seropositive for Western equine encephalitis virus, however, antibodies against this virus were reported in *A. jamaicensis* from Haiti (McLean *et al*. 1979).

A Brazilian study using the complete genomic sequencing of coding genes of Tamana bat virus detected low but significant similarity scores between this virus's sequences and that of other flaviviruses and also in the amino acid sequences of structural and nonstructual genes. It was isolated from salivary glands and spleens of *Molossus ater* and *T. brasiliensis* from Trinidad as well (Price 1978a). Tamana bat virus appears to constitute a distinct genetic group that is genetically not closely related to any other reported flaviviruses. The weakest identity scores were found in part of the RNA-dependent RNA polymerase. Some Taman proteins are first present as polyproteins

which must be cleaved before becoming active. Cleavage sites of the Tamana polyprotein by the virus protease, however, are substantially different from other "classical" flaviviruses. Tamana virus has itself shows extremely significant genetic divergence (de Lamballerie *et al*. 2002).

6.3.2.7 Hepaciviruses and pegiviruses Hepaciviruses and pegiviruses are other genera of flaviviruses. GB virus B is a member of the Hepacivirus genus and, following experimental exposure, infects New World monkeys and causes clinical hepatitis. Hepaciviruses are also found in dogs and horses. The pegivirus genus includes GB viruses -A, -C, and GBV-D. GBV-A virus is present in nonhuman primates, but not humans, while GBV-C frequently infects humans and chimpanzees. GBV-D is present in Old World frugivorous bats. None of the pegiviruses are known to be pathogenic (reviewed by Quan *et al*. 2013). In a survey of oral or rectal swabs, sera, kidney, liver, or lung samples of 1615 apparently healthy bats from eight families, 44 genera, and 58 species and seven countries detected viral RNA in six of the bat families. This study discovered three new species of hepaciviruses and nineteen new pegiviruses in bats (Quan *et al*. 2013). The prevalence of infection in the tested bats was 0.6% for bat hepaciviruses and 4% for pegiciviruses. Despite a lack of evidence of viral recombination, viruses from the African bats (*Mops condylurus, O. martiensseni*, and *Taphozous* species) as well as *Pteropus giganteus* from Bangladesh had coinfections with different clades of pegiviruses and one bat harbored a hepacivirus and a pegivirus. These coinfections suggest a potential for recombination to occur (Quan *et al*. 2013).

6.3.2.8 Other flaviviruses Montana *myotis* leukoencephalitis virus is a flavivirus isolated from bats in the western US. In severely immunocompromised mice, infection causes a fatal encephalitis, but immunocompetent mice do not support infection. It falls within the clade of flaviviruses with no known vector, which includes Rio Bravo, Modoc, and Apoiviruses (Charlier *et al*. 2002). Infection with Rio Bravo virus leads to clinical disease in humans which includes systemic or central nervous system illness along with orchitis or oophoritis. Rio Bravo virus was found in salivary glands of *T. brasiliensisas* in the southern US in 3.4% of the adult bats ($n = 1075$), but only one suckling ($n = 200$) and no fetuses. It was not detected in brains, mammary glands, kidneys, or lungs of infected bats (Constantine & Woodall 1964).

A survey of five flaviviruses was conducted in Guatemala ($n = 332$). From the serum of 38% of 42 species of bats from 13 sites, 26% of the bats had antibodies against at least one flavivirus (Ubico & McLean 1995). Rio Bravo virus antibodies were detected in 19% of the bats. *Artibeus* species bats had the highest prevalence of SLE virus (4.5% of all tested bats), although *G. soricina* was also positive. Six bat species were seropositive for equine encephalitis virus: *A. jamaicensis, Artibeus literalis, G. soricina, Rhynchonycteris naso*, and *S. lillum*. Antibodies against Western equine encephalitis virus were only found in *A. lituratus* (Ubico & McLean 1995). Six bat species were seropositive for VE virus: *A. jamaicensis, C. brevicauda, C. subrufa, P. discolor, S. lillum*, and *Sturnira ludovlci*. The species with the highest prevalence was *A. jamicensis* (Ubico & McLean 1995). Interestingly, the frequency of anti-VE virus antibodies was much lower in bats in Guatemala than in other mammals and varied within host species between localities and years (Seymour *et al*. 1978a).

Kyasanur Forest disease virus is the tick-borne causative agent of Kyasanur Forest disease in humans and nonhuman primates in India. This disease was discovered in 1957 and manifests as a severe hemorrhagic fever with a 3.4% fatality rate in humans. An average of 400–500 human cases occur yearly. Short-term neurological manifestations may occur and include severe headache, mental disturbance, tremors, rigidity, photophobia, and eye pain without evidence of meningitis or encephalitis (reviewed by Holbrook 2012). Antibodies to this virus have been found in the frugivorous *Rousettus leschenaultia* (Pavri & Singh 1965) and *Cynopterus sphinx* (Pavri & Singh 1968) as well as the insectivorous *Rhinolophus rouxi* and *Ornithodoros* bat ticks (Rajagopalan *et al*. 1969).

6.3.3 Hepeviruses

Hepatitis E virus is a nonenveloped, spherical, ssRNA (+) member of the Hepeviridae family. It replicates in hepatocytes and causes self-limiting mild to severe acute viral hepatitis in young adults and has a mortality rate of 28% during pregnancy. Hepatitis E virus is classified into four genotypes: 1 and 2 are found in tropical regions, transmitted via contaminated food or water, and more prone to cause severe disease. Genotypes 3 and 4 are found in temperate areas and transmitted by contact with pigs or deer and, sometimes, cattle, sheep, and horses. Hepatitis E is considered to be an emerging disease in Europe and North America (reviewed by Drexler *et al*. 2012). Other distinct Hepeviridae lineages exist in rats, chickens, and trout.

In order to characterize hepatitis E viruses in bats and their relationship to human viruses, almost 4000 specimens from 85 bat species from five continents were screened for the presence of hepatitis E virus RNA. Viruses were found in 0.18% of tested bat sera (at low concentrations) and feces and liver (at high concentrations) in animals from western Africa, Central America, and Europe. These hepeviruses formed a new, distinctive, and highly diverse clade. Bats in which hepevirus RNA was detected only in fecal samples are as follows: *Hipposideros abae*, Noack's roundleaf bat (*Hipposideros cf. ruber*), Bechstein's bat (*M. bechsteinii*), Daubenton's myotis (*M. daubentonii*), and Geoffroy's bat (*M. emarginatus*). Hepatitis E virus RNA was also found in the liver of *E. serotinus* and the blood of the great striped-faced bat (*Vamipyrodes caraccioli*). A study of over 90 000 human blood samples found no evidence for bat-to-human transmission (Drexler *et al*. 2012).

Only two species of indigenous mammals reside in New Zealand – the lesser short-tailed bat (*Mystacina tuberculata*) and the long-tailed bat (*Chalinolobus tuberculatus*). RNA analysis of the virome of the former found a novel species of hepevirus, named the New Zealand hepevirus. It shares 30.3% amino acid identity with its closest relative, the cut-throat trout virus (J. Wang *et al*. 2015).

6.3.4 Picornaviruses

Picornaviruses are small, nonenveloped, positive-sense single-stranded RNA viruses found in a wide variety of animals, including humans. They can cause severe respiratory, cardiac, hepatic, neurological, mucocutaneous, and systemic diseases, including the common cold; hand, foot and mouth disease; conjunctivitis; aseptic meningitis; encephalitis; myocarditis; and hepatitis. A novel picornavirus was isolated from fecal

samples of healthy *M. schreibersii* in Hungary in 2013. This bat picornavirus belongs to the *Mischivirus* genus (Kemenesi *et al*. 2015). A survey of 1108 bats in Hong Kong discovered RNA from three picornaviruses (groups 1, 2, and 3) in alimentary specimens of 12 bats from five species representing four genera. Picornavirus prevalence in positive bats is as follows: 1.5% of *H. armiger* ($n=68$), 2.6% of *M. pusillus* ($n=78$), 3.2% of *M. schreibersii* ($n=222$), 1.6% of *P. abramus* ($n=61$), and 0.3% of *R. sinicus* ($n=309$). These viruses formed three distinct clusters within the *Picornaviridae* family and had low homology to other picornaviruses (Lau *et al*. 2011). Interestingly, group 2 picornaviruses were found in *M. schreibersii, M. pusillus*, and *P. abramus*, while group 3 picornaviruses were present in *H. armiger* and *R. sinicus*, indicating that bat picornaviruses can cross species- and perhaps genus-barriers among bats.

6.4 BALTIMORE CLASS V VIRUSES

6.4.1 Bunyaviridae

The Bunyaviridae family, with greater than 350 isolates, is the largest family of RNA viruses. This family is divided into five genera (Orthobunyavirus, Phlebovirus, Nairovirus, Hantavirus, and Tospovirus) found throughout the world. Four of the genera infect animals, including humans, and lead to severe disease, including encephalitis, hepatitis, and hemorrhagic fever. Members of the Tospovirus genus only infect plants. Most bunyaviruses are transmitted between susceptible vertebrate hosts and hematophagous arthropods, such as ticks, mosquitoes, and phlebotomine flies. Members of the *Hantavirus* genus, however, do not use insect vectors but are maintained in nature through persistent infection of rodents and acquired by other vertebrates via inhalation of aerosolized, dried excreta, including feces, urine, and saliva (reviewed by Freiberg *et al*. 2014).

Despite having a wide range of virion structures, typical bunyaviruses possess an enveloped spherical to pleomorphic morphology whose diameters are 80–120 nm. Two surface glycoproteins form outward spikes that protrude from the envelope. Hantaviruses arrange their glycoprotein spike complex in a square-shaped assembly. Bunyaviruses are unusual in that they lack a matrix protein (reviewed by Freiberg *et al*. 2014). The Bunyavirus group utilizes a tripartite single-stranded negative-sense RNA genome with S (small), M (medium), and L (large) segments.

6.4.1.1 Nairoviruses The *Nairovirus* genus of arthropod-borne bunyaviruses includes the important emerging human pathogen, Crimean-Congo hemorrhagic fever virus, which causes hemorrhage, shock, and multiorgan system failure with a fatality rate of 3–30%. *Nairovirus* also includes Nairobi sheep disease virus, linked to hemorrhagic gastroenteritis in sheep and goats, with a mortality rate of up to 90% (reviewed by Walker *et al*. 2015). The *Nairovirus* genus clusters into three groups, two of which have been found in bats. The first of these groups infects Microchiroptera and contains Kasokero, Yogue, and Leopards Hill viruses. The former two viruses are present in the Egyptian fruit bat (*Rousettus aegyptiacus*) in Uganda and Senegal, respectively. When inoculated into mice, Kasokero virus causes limb paralysis and kills suckling and adult mice, including mothers of the infected sucklings, suggesting that the

virus may be transmitted via milk (Kalunda *et al*. 1986). Accidental infection of four laboratory workers resulted in mild to severe disease, characterized by fever, headache, abdominal pain, diarrhea, and severe muscle and joint pain (Kalunda *et al*. 1986). Leopards Hill virus is found in leaf-nosed bats (*Hipposideros gigas*) in Zambia. The other group of narioviruses infects Megachiroptera and contains Keterah virus, Issyk-Kul virus, and Gossas virus. Keterah virus was isolated from a tick (*Argae pusillus*) on a lesser Asian yellow house bat (*S. kuhlii*) from Malaysia. Issyk-Kul virus infects common noctule bats (*N. noctula*) in Kyrgyzstan as well as *Myotis blythii, Vespertilio serotinus*, and *Vespertilio pipistrellus*. Gossas virus infects *Tadarida* species bats in Senegal (Walker *et al*. 2015). Kasokero and Issyk-Kul viruses are able to infect humans, resulting in headache, diarrhea, and muscle and joint pain.

6.4.1.2 Orthobunyaviruses Kaeng Khoi virus of the genus *Orthobunyavirus* was isolated from dead *Chaerephon plicata* insectivorous bats from Thailand and Cambodia (Neill 1985; Osborne *et al*. 2003). Brain tissue and brain supernatants from 12 dead bats, as well as from 24 apparently healthy bats, were inoculated into mice. While brain material from the healthy bats did not cause disease in mice, material from 11 of 12 dead bats caused encephalitis in 80–100% of the mice and 67% of mice infected orally subsequently died (Osborne *et al*. 2003). Unlike many of the RNA viruses infecting bats, Kaeng Khoi virus showed very little genetic diversity over the course of 30 years (less than 4% variation) (Osborne *et al*. 2003). Kaeng Khoi virus is able to infect humans, as evidenced by the presence of virus-neutralizing antibodies in 29% of bat guano collectors in a specific bat cave in Thailand (Neill 1985). Of note, the Cambodian cave from which the dead bats were collected is a tourist site, thus Kaeng Khoi virus is a potential public health threat.

6.4.1.3 Hantaviruses Hantaviruses are responsible for two severe to fatal diseases in humans – hemorrhagic fever with renal failure (HFRS) and hantavirus pulmonary syndrome (HPS). Between 60 000 and 150 000 cases of the former occur yearly, with the majority in Asia, particularly China. The hantaviruses which cause HFRS include Hantaan virus in Asia and Europe and Dobrava virus in the Balkans. A less severe form of the disease is caused by Seoul virus worldwide; Puumala virus in Scandinavia; and Bayou, Black Creek Canal, Monongahela, and New York viruses in North America. HFRS is characterized by high fever, severe abdominal or lower back pain, hemorrhagic symptoms, and renal dysfunction, leading to fluid accumulation in the lungs. Hemorrhagic manifestations include severe hemorrhaging and disseminated intravascular coagulation. The fatality rate for HFRS is 5–15% (reviewed by Beltz 2011).

HPS is caused by Sin Nombre virus in North America and the Andes and by Laguna Negra, HU39694, Lechiguanas, Oran, and Juquitiba viruses in South America. Relatively nonspecific symptoms are followed by the cardiorespiratory phase in which fluid enters the alveoli of the lungs, hypoxia, tachypnea, and tachycardia. Death may occur rapidly due to hypoxia or circulatory collapse, high systemic vascular resistance, hypotension, or shock. The fatality rate of HPS is 20–40% (reviewed by Beltz 2011).

Evidence suggests that the original hosts for hantaviruses were either rodents or shrews and moles. Rodents shed virus in their saliva, urine, and feces. Since apparently healthy bats also harbor a diverse population of hantaviruses near the base of the phylogenic tree, they may serve not only as additional reservoir hosts but perhaps also as

ancestors of rodent hantaviruses (Guo *et al*. 2013). Initially believed to be confined to insectivorous bats, hantavirus infection of other trophic species of bats has also been reported. Hantaviruses have been shown to undergo cross-species transmission as well as genetic reassortment (Zhang 2014). Bat hantaviruses are more closely related to those found in shrews and moles from Southeast Asia than those in rodents (Weiss *et al*. 2012), but approximately 50% of shrews and moles harbor hantaviruses, far exceeding the number of these viruses in bats in cumulative studies (Gu *et al*. 2014). This may be due, at least in part, to bats being less susceptible to hantavirus infection or to their innate immune responses that control viral replication and persistence.

Hantaviruses and hantavirus RNA were first isolated from lung and kidney tissues of seropositive *E. serotinus* and *R. ferrumequinum* bats in Korea (Kim *et al*. 1994). These isolates were very closely related to Hantaan virus of humans. Xuan Son virus RNA was detected in lung tissue from 20% ($n=5$) of Pomona roundleaf bats (*H. pomona*) in Vietnam. Lower levels of viral RNA were also present in the liver, kidney, and spleen (Arai *et al*. 2013). Viral RNA is also present in some specimens of bats' intercostal muscles or intestine, rectal swab, or feces (Gu *et al*. 2014). Xuan Son virus appears to share a common ancestry with Magboi virus. Several bat-borne hantaviruses have been found in China: Longquan virus from 25% of *Rhinolophus monoceros* ($n=4$), 23.1% of *R. affinis* ($n=26$), and 2.2% of *Rhinolophus sinicus* ($n=135$); Huangpi virus from *P. abramus*; and Laibin virus from lung tissue of the black-bearded tomb bat (*T. melanopogon*) (Guo *et al*. 2013; Xu *et al*. 2015). The genome of the latter virus has been completely sequenced (Xu *et al*. 2015). Interestingly, no hantavirus RNA sequences were found in *R. pusillus* ($n=250$) or *Rhinolophus pearsonii* ($n=29$) (Guo *et al*. 2013).

Elsewhere, Mouyassué virus is present in the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire (Sumibcay *et al*. 2012) and Magboi virus is found in lung tissue of the hairy split-faced bat (*Nycteris hispida*) in Sierra Leone (Weiss *et al*. 2012). In South America, RNA of another hantavirus, Araraquara virus, was found in two *Diphylla ecaudata* (hematophagous) and one *Anoura caudifer* (nectivorous) bats in Brazil, demonstrating the wide geographical range of hantaviruses in bats as well as demonstrating the presence of hantaviruses in non-insectivorous bat species. The infected bats were captured from an area that had undergone severe ecological disturbances (deforestation and flooding for construction of a dam) that increased bat–human interactions (de Araujo *et al*. 2012). Of note, no hantavirus RNA was found in 111 insectivorous bats from five species in the US or Bolivia (Sumibcay *et al*. 2012). In a more recent study of bat-borne hantaviruses in Brazil, virus-specific antibodies to Araraquara virus were found in 17% of tested bats ($n=53$), five of these were frugivorous, one was carnivorous, and three were hematophagous phyllostomid bats (Sabino-Santo Jr *et al*. 2015). Hantavirus prevalence in bats in that region was greater than that found in rodent reservoirs. Further studies examining the presence of hantavirus in other mammal groups is greatly needed.

6.4.1.4 Phlebotomus viruses This *Bunyavirus* group is divided into *Phlebotomus* fever viruses, transmitted by sandflies and the Uukuniemi group, transmitted by ticks. The following members of this viral group cause human disease: Alenquer, Candiru, Charges, Naples, Punta Toro, Rift Valley fever, Sicilian, Toscana, and severe fever with thrombocytopenia syndrome virus and Heartland viruses (reviewed by Freiberg *et al*. 2014). *Rousettus leschenaultii* bats in western India harbor a phlebotomus virus,

Malsoor virus, that appears to be closely related to severe fever with thrombocytopenia syndrome virus and Heartland virus of humans (Mourya *et al*. 2014).

6.4.2 Orthomyxoviruses

Influenza viruses belong to the family Orthomyxoviridae and have been a major cause of human morbidity and mortality. There exist three (or four) types of influenza virus – A, B, C, and possibly D. Influenza A viruses circulate through waterfowl or other birds and then use a mammalian host, typically a pig, as a "mixing vessel" for viral reassortment. Group A viruses appear to have the greatest risk of generating severe human pandemics, including the H1N1 "Spanish influenza" of 1918. Recently, however, the role of other mammals in interspecies transfer of severe infections has begun to be appreciated. For example, highly pathogenic avian H5N1 viruses are able to cause natural infection and disease in domestic cats and H3N8 equine influenza A, upon entry into the dog population, evolved into a lineage of canine influenza A virus responsible for severe respiratory disease. Influenza B viruses, formerly believed to exclusively infect humans, are also able to use harbor seals and guinea pigs as hosts (reviewed by Poole *et al*. 2014).

Two viral surface proteins are vital to the influenza virus lifecycle – the hemagglutinin (HA) and neuraminidase (NA) glycoproteins. They also are the major antigenic targets for vaccines. HA is responsible for virus binding and attachment to the host cell plasma membrane, cell entry via endocytosis, and fusion with endosomes. The cellular receptor for HA is the α2,6 sialic acid (SA)-linkage galactose receptor in mammals and the α2,3 SA-linkage galactose receptor in birds.

Influenza A has produced several severe human pandemics in addition to the 1918 Spanish influenza. These are the 1957 ("Asian flu"), and 1968 ("Hong Kong flu"), as well as the relatively mild pandemic of 2009–2010 ("H1N1 swine flu"). The 1918 influenza led to arguably the worst single pandemic in human history, and was responsible for the deaths of at least 20–40 million people over several years. Extremely lethal avian influenza viruses have killed large numbers of birds in recent years and, despite a lack of human-to-human transmission, have a high mortality rate in those few humans who became infected from an avian host. Adaptive mutations in the viral RNA-dependent RNA polymerase gene are believed to have been at the root of these pandemics by allowing influenza viruses of different mammals to overcome the species barrier, followed by recombination with influenza viruses of the host species.

Bats also host influenza A viruses, as was evidenced by isolation of influenza HA and NA proteins of the Hong Kong complex (H3N2 influenza) from lungs and tracheal samples of the insectivorous *N. noctula* bats inoculated into chick embryos (L'vov *et al*. 1979). No viable virus, however, has been propagated in the embryos or cell lines. The two viral surface proteins do not appear to belong to the A, B, C, or recently proposed D bovine and swine types of influenza viruses. The internal proteins, however, are similar to those of other influenza viruses (Brunotte *et al*. 2016). Interestingly, nonstructural protein 1 (NS1) from the H17N10 and H18N12 bat viruses described below is able to antagonize the human interferon response in a process dependent upon RNA binding (Turkington *et al*. 2015). A recent report detected antibodies to influenza A H9 glycoprotein in the sera of 30% of healthy tested *E. helvum* bats in Ghana ($n = 100$) (Freidl *et al*. 2015). Of note, H9N2 is the most

common bird influenza virus and may also cause mild illness in humans. Seropositivity of the bats did not correlate with age, gender, or season of the year.

An influenza A virus with a novel HA was found in 10% of the rectal swabs of the frugivorous *S. lilium* from Guatemala ($n=29$) (Tong *et al*. 2012). Viral RNA was also present in intestine, liver, lung, and kidney tissue samples, but not oral swabs. This new subtype of HA, designated H17, has approximately 45% amino acid sequence identity to those from all other known influenza A subtypes but appears to be more closely related to group 1 (H1, 2, 5, 6, 8, 9, 11, 12, 13, and 16) than group 2 subtypes (Tong *et al*. 2012). Unlike HA and internal genes, the NA gene of this bat influenza virus is extremely divergent from that of known influenza viruses (N1–N9 and the NA of influenza B viruses) and is accordingly named N10. The presence of influenza A in frugivorous or nectivorous bats is important since these bats may have close contact with birds feeding on the same flowering plant.

In 2013, RNA from a second bat influenza A virus, designated H18N11, was detected in rectal swabs and intestinal tissue from a flat-faced fruit bat (*A. planirostris*) from the Amazon River Basin of Peru. A number of other Peruvian bat species were subsequently found to be seropositive for the H18 protein (*A. lituratus, Artibeus obscurus, A. planirostris, C. brevicauda, Carollia perspicillata, D. rotundus, M. molossus, P. discolor, Phyllostomus hastatus, Platyrrhinus recifinus, Rhinophylla pumilio*, and *Vampyressa bidens*). The polymerase gene from the Peruvian bats was most closely related to that from the H17N10 Guatemalan bat influenza virus (Tong *et al*. 2013). Interestingly, phylogenetic analyses indicated that, for some influenza gene segments, New World bats contain more diversity than has been found in all other host species combined, suggesting a long-term bat-virus association.

H17N10 and H18N11 are the only known influenza A bat viruses. They infect different genera of bats and were isolated from locations over 3000 km apart. They have not, however, been reported outside of South and Central America. An extensive search for H1–H16 and H17-related RNA from 1369 Central European bats from 31 locations and from 26 species of the Vespertilionidae and Rhinolophidae families failed to find any evidence for this gene and, by inference, influenza A viruses (Fereidouni *et al*. 2015).

H17's crystal structure was determined and was found to be unusual in its high degree of susceptibility to trypsin and low thermostability (50% unfolded at 37 °C). Bats are more equipped as hosts for the H17 viruses since their temperature drops during hibernation and H17 is more resistant to lower temperatures. Importantly, H17 has a distorted SA host binding site that may be responsible for the inability of these viruses to bind mammalian or bird SA receptors (Sun *et al*. 2013). H17's overall structure is similar to that of other influenza HAs and the receptor-binding site contains conserved aromatic residues that form the base of the host cell binding site. Its amino acid similarity to the other HAs is comparable with that found among the other HAs as well. Other components of the binding site, however, contain substitutions in conserved residues, leaving the site highly acidic, thus unlikely to bind to the negatively charged sialylated host cell receptors (Zhu *et al*. 2013). Accordingly, H17 does not permit bat viral entry into dog kidney cells (Sun *et al*. 2013). Together, this suggests that H17-bearing influenza viruses may use an alternative host cell binding pathway than that used by either other mammals or birds (Sun *et al*. 2013; Zhu *et al*. 2013).

NA is a neuraminidase whose function is to remove the terminal sialic acid from the host cell receptor. This is necessary to prevent aggregation of the progeny virions as they

exit the host cell during the final stage of cellular infection as well as allowing their release from the lysed target cell. Influenza A NAs from birds and mammals, with the exception of bats, are divided into group 1 (N1, N4, N5, and N8) and group 2 (N2, N3, N6, N7, and N9). The amino acid sequences of influenza A and B NAs differ by 75%, yet they have similar topology and share a highly conserved active site. The bat influenza N10 contains the canonical sialidase fold but without the corresponding sialidase activity, perhaps because most of the required functional amino acid residues have been substituted (Li *et al*. 2012). The putative active site is also much wider than in other NA proteins because two of the protein loops have been displaced (Zhu *et al*. 2012). N10 also contains an atypical 150-loop that is involved in intermolecular polar interactions between N10 molecules of the N10 tetramer (Li *et al*. 2012). It has only 20–27% identity with NAs from other influenza A viruses (Zhu *et al*. 2012). The findings that the N10 protein exhibits low to no NA activity in addition to its structural changes suggest that it may serve a different purpose than other NA proteins (Li *et al*. 2012; Zhu *et al*. 2012). As is the case for H17N10, the hemagglutinin and neuraminidase of H18N11 do not use sialic acid as a ligand for viral attachment or as a substrate during virion release, suggesting that they use a mode of viral attachment and membrane fusion that is unique to bat influenza viruses.

Kidney cells from the nectivorous *P. alecto* bats can be experimentally infected with and sustain replication of human H1N1 and H5N1 viruses (type A influenza) (Dlugolenski *et al*. 2013). Co-infection of these cells with influenza A viruses from humans and pigs also results in novel reassortments, as happens during the sometimes severe bird–pig–human influenza outbreaks. Influenza viruses mutate much more readily than most RNA viruses and can produce adaptive mutations in the HA and NA genes that allow the viruses to accommodate differences in different host species' sialoglycoproteins. A recent study exposed a variety of diverse bat cell lines (derived from fetal or adult bat kidneys or lungs from New and Old World bats) to a prototypical human influenza A virus (Poole *et al*. 2014). A specific mutation in the human influenza virus polymerase at residue 285 allowed the altered human viruses to efficiently replicate in all of the tested bat cell lines and to produce highly cytopathic progeny. This residue was not previously known to be used in adaptive mutation, although an adaptive E627K mutation in the polymerase residue has been known to allow avian influenza to overcome a protective barrier to replication in human cells. Bat-origin influenza viruses use S627 instead. In contrast to the case found in the polymerase gene, which contained seven of the eleven adaptive mutations, this study did not detect adaptive mutations in the HA or NA genes (Poole *et al*. 2014).

6.4.3 Arenaviridae

Arenaviruses are ssRNA (−) viruses with a bi-segmented (L and S) genome. The family contains several pathogenic species of hemorrhagic fever viruses with fatality rates of approximately 30%. Species known to be pathogenic to humans include Junín, Sabiá, Guanarito, and Machupo viruses, causing Argentine, Brazilian, Venezuelan, and Bolivian hemorrhagic fevers in South America. Tacaribe virus belongs to the same New World group of arenaviruses but is not known to cause human disease naturally, although an accidental laboratory infection resulted in flu-like symptoms. Lassa virus, from the Old World group of arenaviruses, causes severe to fatal hemorrhagic fever in West Africa. Arenaviruses are found in and transmitted to humans by rodents, with the exception of Tacaribe virus.

Several species of bats in Trinidad are seropositive for Tacaribe virus: *A. lituratus* and *A. jamaicensis trinitatis* (Downs *et al*. 1963; Ubico & McLean 1995). Tacaribe neutralizing antibodies have additionally been found in *S. lilium* and *Vampyrops helleri* (fruit bats) and in *D. rotundus* (hematophagous). In order to examine the possibility that bats serve as a reservoir host for this virus in humans, *A. jamaicensis* bats were experimentally infected with Tacribe virus (Cogswell-Hawkinson *et al*. 2012). Bats receiving low dose inoculums developed asymptomatic infection, followed by viral clearance, while many of the animals receiving high levels of virus developed morbidity (pneumonia, poor responses to mechanical stimuli, tremors, incoordination, and inability to fly) and pathologic changes were found in livers, spleens, and brains. Almost all of the animals receiving high doses of virus ($n=12$) either died or were euthanized upon becoming moribund (Cogswell-Hawkinson *et al*. 2012). Virus was not passed from infected to control bats. In a separate study, Tacaribe-specific antibodies were not found in sera from 29 humans, 20 of which came from bat collectors (Price 1978b). These findings suggest that *A. jamaicensis* bats might not be a reservoir host for Tacribe virus since they do not support a persistent and nonlethal infection, despite the fact that some of the bats intermittently shed virus (viral RNA) orally or rectally (Cogswell-Hawkinson *et al*. 2012).

6.5 LARGE, MULTI-VIRUS STUDIES

A large study in Trinidad from 1972 and 1974 detected hemagglutination inhibition antibodies against at least one of twelve viruses in bats (Price 1978b). The bat species testing positive in this assay are as follows: Phyllostomidae family members *Anoura geoffroyi, Artibeus cinereus, A. jamaicensis, A. lituratus, C. perspicillata, G. soricina, P. hastatus*, and *S. lilium*; Molossidae family members *M. ater, Molossus molossus* (*Natalus tumidirostris*), and *T. brasiliensis*; Emballonuridae family member *T. melanopogon*; and Mormoopidae family members *Pteronotus davyi, P. parnelli*; and *V. helleri*. Only a few bat species produced these antibodies against Mucambo, eastern equine encephalitis, Oriboca, Restan, Manzanilla, Guama, Bimiti, and Catu viruses. In contrast, hemagglutination inhibition antibodies were detected against the following viruses in almost all of the antibody-positive bats: Ilheus, SLE, DENV-2, and yellow fever viruses. The same study detected fully or partially protective neutralizing antibodies against Tacaribe virus in *A. jamaicensis, A. lituratus, P. hastatus, S. lilium, Tonatia bidens, Uroderma bilobatum*, and *V. helleri*. For a complete report of all bat and viral species tested and the results, see Price (1978b).

6.6 CONCLUSIONS

Baltimore Class III reoviruses infect a wide range of vertebrates, invertebrates, and plants. Accordingly, a number of reoviruses have been discovered in bats (several orbiviruses, rotaviruses, bat MRV, Nelson Bay virus; Pulau virus; Xi River virus, and Broome virus). These bat viruses have not been shown to infect humans, even though some of them might induce antibodies that cross-react with related human orthoreoviruses. This may be the case for the bat Pulau virus and the human Melaka virus. Related, pathogenic human orthoreoviruses have not been isolated from bats.

Baltimore Class IV contains several viral groups that infect bats, including astroviruses, hepeviruses, picornaviruses, and flaviviruses. Astroviruses infect many mammalian species. Their prevalence in various bat species, based on anal swabs, ranges from 25% to greater than 90%. As is the case for other findings based on viral RNA or viruses from anal swabs, it is difficult to determine whether these findings are indicative of infection or merely reflect viral passage through the digestive tract, especially in light of the facts that almost all of the positive bats in this study were insectivores or carnivores and even frugivorous bats are known to ingest insects while feeding.

Two members of the hepevirus group have been reported in bats. The first of these is the human pathogen, hepatitis E virus, which causes severe acute viral hepatitis in young adults or pregnant women. A very large study of 85 bat species from five continents only found hepatitis E virus RNA in less than 0.2% of bat sera or in feces and liver. These viruses belonged to a new and distinctive viral clade. Examination of 90 000 human blood samples found no evidence of zoonotic transmission of this virus from bats. Pigs, however, have strongly been implicated as a major source of human infection. The New Zealand hepevirus is the second member of this viral group from bats. Its closest relative is the cut-throat trout virus.

Picornaviruses infect a wide range of animals, including humans and bats, and cause mild diseases in humans (the common cold) to severe respiratory illness (respiratory, cardiac, hepatic, and neurological diseases). A picornavirus was found in fecal samples of *M. schreibersii* from Hungary and three other picornaviruses in alimentary specimens of five species of bats from Hong Kong. They form three distinct clusters with low homology to other picornaviruses.

Flaviviruses detected in bats include dengue viruses; Venezuelan, Japanese, and St. Louis encephalitis viruses, WNV, Kyasanur Forest viruses, hepaciviruses, pegiviruses, and Montana *myotis* leukoencephalitis, some of which are human pathogens. All four dengue serotypes are present in bats in Latin America and dengue virus has been detected in bat heart tissue. Experimental infection of bats with several dengue serotypes failed to find viral replication or persistent infection, calling into question whether bats serve as significant viral reservoirs.

Antibodies to VE virus are present in several bat species. Following experimental infection, the resulting viremia was high enough to subsequently infect the *Culex* mosquito vector. High levels of viremia were present in 56% of oropharyngeal cavities of bats, however in few urine or fecal samples. Whether the results with experimentally infected bats are indicative of the situation in naturally infected bats is unknown.

Pigs and waterfowl are known viral reservoirs for JEV. Viral isolates and antibodies against the virus have been found in bats, but no viral RNA has been found in the brain or liver samples. Naturally infected bat populations had equal frequencies of isolation and neutralizing antibodies throughout the year, however, human infections are highest in the summer. Virus was only found in June–late July in mosquitoes and in bird and pig reservoir hosts in late July–early August, suggesting that bats or other mammalian species may sustain the virus population during the remainder of the year. Additionally, virus was isolated from bat blood and brown fat from several locations in Japan but not in bats from northern Japan in a region with few human cases. When *P. alecto* flying foxes were experimentally infected with the virus by bites of infected *Culex* mosquitoes, all bats remained asymptomatic. IgG was nevertheless present in 60% of the bats and they were able to infect recipient mosquitoes, even in the absence

of detectable viremia. This could be due to viral replication in the bats' skin at the bite site in the absence of virus presence in the blood.

WNV and SLE virus flaviviruses use mosquitoes as vectors and birds serve as the principal hosts. Humans and horses are also infected and, rarely, develop severe disease. While antibodies were present in several naturally or experimentally infected bat species, these antibodies are known to cross-react with other flaviviruses. Very few WNV antibody-positive bats were found in an endemic area of the southern US, leading Davis *et al*. (2005) to conclude that bats are unlikely to act as amplifying hosts for WNV. However, multiple strains of SLE virus were isolated in *T. brasiliensis* bats during two human epidemics. This virus was present in bats nearly year-round, including times in which no virus was detectable in mosquitoes, birds, or humans, opening the possibility that *T. brasiliensis* may act as a persistent reservoir host between human outbreaks.

Several other pathogenic human flaviviruses have been detected in bats. Tick-borne Kyasanur Forest disease causes severe hemorrhagic fever in humans. Several species of frugivorous or insectivorous bats have antibodies to the causative virus. Rio Bravo virus has been detected in less than 4% of bat salivary glands in the southern US. This virus causes systemic or central nervous system illnesses in humans. Members of the Entebbe bat virus group were isolated from bat salivary glands, spleen, and lungs in Uganda. It caused illness in several laboratory workers. It is most closely related to the nonpathogenic Sokoluk and Yokose viruses isolated from bats in Japan, Southeast Asia, and the Philippines.

Other flaviviruses detected in bats are not associated with disease in bats or humans, including Taman bat virus in Latin American bats. Six bat families harbor hepaciviruses, pegiviruses, or both. Montana *myotis* leukoencephalitis has also been reported in bats from the western US.

Baltimore Class V viruses associated with bats include bunyaviruses, orthomyxoviruses, and arenaviruses. The bunyaviruses include nairoviruses, orthobunyaviruses, hantaviruses, and phlebotomus viruses. Nairoviruses are arthropod-borne bunyaviruses that include the human pathogen, Crimean-Congo hemorrhagic fever virus, which causes a very severe, life-threatening disease. Two of the three groups of nairoviruses are found in bats. The first of these is found in Microchiroptera and is composed of Kasokero, Yogue, and Leopards Hill viruses. Accidental infection of four laboratory workers with Kasokero virus led to mild to severe disease. The other group of nairoviruses infects Megachiroptera and is composed of Keterah, Issyk-Kul, and Gossas viruses. Kasokero and Issyk-Kul viruses cause mild disease in humans, including headache, diarrhea, and muscle and joint pain.

The orthobunyavirus Kaeng Khoi virus has been isolated from brains of both healthy and dead bats. While experimental infection of mice with brain material from healthy bats was not pathogenic, material from dead bats caused severe to fatal encephalitis. Guano collectors from the cave where the dead bats were collected developed neutralizing antibodies to Kaeng Khoi virus in the absence of apparent illness.

Hantaviruses cause two very severe to fatal diseases in humans, hemorrhagic fever with renal failure and hantavirus pulmonary syndrome. Since rodents shed virus in their excreta, they, or shrews and moles, may have been original hosts as well as the primary reservoirs for hantaviruses. Healthy bats also carry a diverse group of hantaviruses. It has been suggested that bat hantaviruses may have been ancestral to those in rodents. It has also been suggested that bats may also serve as additional reservoir hosts, partially based on their ability to undergo interspecies transmission and genetic reassortment. Bat

hantaviruses, however, appear to be more closely related to those from shrews and moles from Southeast Asia than to those from rodents. While inhalation to aerosolized rodent excreta has been firmly linked to zoonotic infections, it is unclear what route of zoonotic transmission from bats would allow the large numbers of human cases of hemorrhagic fever with renal failure (60 000–150 000) that occur yearly.

Phlebotomus includes several viruses that cause human disease, among them, Rift Valley fever virus from Africa, severe fever with thrombocytopenia syndrome virus from Asia, and Heartland virus from the US. Malsoor virus has been detected in bats from India. While this phlebotomus virus is closely related to the two latter human pathogenic viruses, the geographical ranges of Malsoor and Heartland viruses are quite different.

Influenza viruses are orthomyxoviruses. Members of the viral group A have occasionally led to major pandemics in humans with enormous loss of lives. Influenza viruses have been found in many diverse types of vertebrates, including humans, pigs, birds, domestic animals, and bats. Influenza virus proteins HA and NA are critical to host tropism. Viruses from South or Central American bats carry two new HA proteins, H17 and H18, as well as new NA proteins, N10 and N11, that greatly differ from known influenza viruses of other animal species. Bat HA proteins have a distorted host cell binding site as well as a critical charge difference from other influenza viruses. Additionally, H17 is thermally unstable at human body temperature and is better suited to the lower temperatures found in bats during torpor or hibernation. Bat NA proteins have less than 30% identity with NAs from other influenza A viruses and exhibit very low, if any, typical NA activity. The bat viruses are also unable to bind mammalian or bird host cell receptors, suggesting that they use manners of binding and existing from their cellular targets that are unique to bat influenza viruses. Taken together, these finding make the possibility of zoonotic transfer of bat influenza viruses very unlikely. It should be noted, however, that bat kidney cells can be experimentally infected with human H1N1 and H5N1 viruses and that co-infection permits recombination of these human influenza A viruses in bat kidney cells.

Some arenaviruses are highly pathogenic to humans, including hemorrhagic fever viruses that cause high fatality rates in South America and Africa. The New World Tacaribe virus, which has not been shown to cause natural human disease, has been detected in several species of bats from the area. Experimental infection of bats with low doses of Tacaribe virus was asymptomatic and resulted in viral clearance. Bats receiving high viral dosages either become moribund or died without passing infection to control bats. Additionally, Tacaribe-specific antibodies were not found in sera from 20 bat collectors. These findings suggest that bats are not reservoir hosts for Tacribe virus since they do not support persistent and nonlethal infection.

REFERENCES

Aguilar-Setién A, Romero-Almaraz ML, Sánchez-Hernández C, Figueroa R, Juárez-Palma LP, García-Flores MM, Vázquez-Salinas C, Salas-Rojas M, Hidalgo-Martínez AC, Pierlé SA, García-Estrada C, Ramos C. 2008. Dengue virus in Mexican bats. *Epidemiology and Infection.* 136(12):1678–1683.

Allen R, Taylor SK, Sulkin SE. 1970. Studies of arthropod-borne virus infections in Chiroptera. Evidence of natural St. *Louis encephalitis virus infection in bats. American Journal of Tropical Medicine and Hygiene.* 19(5):851–859.

Arai S, Nguyen ST, Boldgiv B, Fukui D, Araki K, Dang CN, Ohdachi SD, Nguyen NX, Pham TD, Boldbaatar B, Satoh H, Yoshikawa Y, Morikawa S, Tanaka-Taya K, Yanagihara R, Oishi K. 2013. Novel bat-borne hantavirus, Vietnam. *Emerging Infectious Diseases.* 19(7):1159–1161.

Banerjee K, Bhat HR, Geevarghese G, Jacob PG, Malunjkar AS. 1988. Antibodies against Japanese encephalitis virus in insectivorous bats from Karnataka. *Indian Journal of Medical Research.* 87:527–530.

Beltz LA. 2011. *Foundations of Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey-Bass and APHA Press: San Francisco, CA.

Bunde JM, Heske EJ, Mateus-Pinilla NE, Hofmann JE, Novak RJ. 2006. A survey for West Nile virus in bats from Illinois. *Journal of Wildlife Diseases.* 42(2):455–458.

Brunotte L, Beer M, Horie M, Schwemmle M. 2016. Chiropteran influenza viruses: flu from bats or a relic from the past? *Current Opinion in Virology.* 16:114–119.

Cabrera-Romo S, Recio-Tótoro B, Alcalá AC, Lanz H, del Ángel RM, Sánchez-Cordero V, Rodríguez-Moreno Á, Ludert JE. 2014. Experimental inoculation of *Artibeus jamaicensis* bats with dengue virus serotypes 1 or 4 showed no evidence of sustained replication. *American Journal of Tropical Medicine and Hygiene.* 91(6):1227–1234.

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. 2006. Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews.* 19:531–545.

Charlier N, Leyssen P, Pleij CW, Lemey P, Billoir F, Van Laethem K, Vandamme AM, de Clercq E, de Lamballerie X, Neyts J. 2002. Complete genome sequence of Montana Myotis leukoencephalitis virus, phylogenetic analysis and comparative study of the 3′ untranslated region of flaviviruses with no known vector. *Journal of General Virology.* 83(Pt 8):1875–1885.

Chu DKW, Poon LLM, Guan Y, Peiris JSM. 2008. Novel astroviruses in insectivorous bats. *Journal of Virology.* 82(18):9107–9114.

Chua KB, Crameri G, Hyatt A, Yu M, Tompang MD, Rosli J, McEachern J, Crameri S, Kumarasamy V, Eaton BT, Wang L-F. 2007. A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. *Proceedings of the National Academy of Science USA*. 104(27):11424–11429.

Chua KB, Voon K, Crameri G, Tan HS, Rosli J, McEachern JA, Suluraju S, Yu M, Wang LF. 2008. Identification and characterization of a new orthoreovirus from patients with acute respiratory infections. *PLoS ONE.* 3(11):e3803.

Chua KB, Voon K, Yu M, Keniscope C, Abdul Rasid K, Wang LF. 2011. Investigation of a potential zoonotic transmission of orthoreovirus associated with acute influenza-like illness in an adult patient. *PLoS ONE.* 6(10):e25434.

Cheng P, Lau CS, Lai A, Ho E, Leung P, Chan F, Wong A, Lim W. 2009. A novel reovirus isolated from a patient with acute respiratory disease. *Journal of Clinical Virology.* 45:79–80.

Cogswell-Hawkinson A, Bowen R, James S, Gardiner D, Calisher CH, Adams R, Schountz T. 2012. Tacaribe virus causes fatal infection of an ostensible reservoir host, the Jamaican fruit bat. *Journal of Virology.* 86(10):5791–5799.

Constantine DG, Woodall DF. 1964. Latent infection of Rio Bravo virus in salivary glands of bats. *Public Health Reports.* 79:1033–1039.

Cui J, Counor D, Shen D, Sun G, He H, Deubel V, Zhang S. 2008. Detection of Japanese encephalitis virus antibodies in bats in Southern China. *American Journal of Tropical Medicine and Hygiene.* 78(6):1007–1011.

Davis A, Bunning M, Gordy P, Panella N, Blitvich B, Bowen R. 2005. Experimental and natural infection of North American bats with West Nile virus. *American Journal of Tropical Medicine and Hygiene.* 73(2):467–469.

de Araujo J, Thomazelli LM, Henriques DA, Lautenschalager D, Ometto T, Dutra1 LM, Aires CC, Favorito S, Durigon EL. 2012. Detection of hantavirus in bats from remaining rain forest in São Paulo, Brazil. *BMC Research Notes*. 5:690.

de Lamballerie X, Crochu S, Billoir F, Neyts J, de Micco P, Holmes EC, Gould EA. 2002. Genome sequence analysis of Tamana bat virus and its relationship with the genus *Flavivirus*. *Journal of General Virology.* 83:2443–2454.

Dlugolenski D, Jones L, Tompkins SM, Crameri G, Wang L-F, Tripp RA. 2013. Bat cells from *Pteropus alecto* are susceptible to influenza A virus infection and reassortment. *Influenza and Other Respiratory Viruses.* 7(6):900–903.

Downs WC, Anderson CR, Spence L, Arfken THC, Greenhall AG. 1963. Tacaribe virus, a new agent isolated from *Artibeus* bats and mosquitoes in Trinidad, West Indies. *American Journal of Tropical Medicine and Hygiene*. 12:640–646.

Drexler JF, Corman VM, Wegner T, Tateno AF, Zerbinati RM, Gloza-Rausch F, Seebens A, Müller MA, Drosten C. 2011. Amplification of emerging viruses in a bat colony. *Emerging Infectious Diseases.* 17(3):449–456.

Drexler JF, Seelen A, Corman VM, Fumie Tateno A, Cottontail V, Melim Zerbinati R, Gloza-Rausch F, Klose SM, Adu-Sarkodie Y, Oppong SK, Kalko EK, Osterman A, Rasche A, Adam A, Müller MA, Ulrich RG, Leroy EM, Lukashev AN, Drosten C. 2012. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. *Journal of Virology.* 86(17):9134–9147.

Du L, Lu Z, Fan Y, Meng K, Jiang Y, Zhu Y, Wang S, Gu W, Zou X, Tu C. 2010. Xi River virus, a new bat reovirus isolated in southern China. *Archives of Virology.* 155:1295–1299.

Dufkova L, Straková P, Širmarová J, Salát J, Moutelíková R, Chrudimský T, Bartonička T, Nowotny N, Růžek D. 2015. Detection of diverse novel bat astrovirus sequences in the Czech Republic. *Vector Borne Zoonotic Diseases.* 15(8):518–521.

Fereidouni S, Kwasnitschka L, Buschmann AB, Muller T, Freuling C, Schatz J, Pikula J, Bandouchova H, Hoffmann R, Ohlendorf B, Kerth G, Tong S, Donis R, Beer M, Harder T. 2015. No virological evidence for an influenza A-like virus in European bats. *Zoonoses and Public Health.* 62:187–189.

Fischer K, Zeus V, Kwasnitschka L, Kerth G, Haase M, Groschup MH, Balkema-Buschmann A. 2016. Insectivorous bats carry host specific astroviruses and coronaviruses across different regions in Germany. *Infection, Genetics and Evolution.* 37:108–116.

Freiberg AN, Bente DA, Le Duc JW. 2014. Bunyaviruses: Hantavirus and others. In: *Viral Infections of Humans*. RA Kaslow, LR Stanberry, and JW Le Duc (eds). Springer: New York, pp. 173–197.

Freidl GS, Binger T, Müller MA, de Bruin E, van Beek J, Corman VM, Rasche A, Drexler JF, Sylverken A, Oppong SK, Adu-Sarkodie Y, Tschapka M, Cottontail VM, Drosten C, Koopmans M. 2015. Serological evidence of influenza A viruses in frugivorous bats from Africa. *PLoS ONE.*10(5):e0127035.

Gard G, Compans RW. 1970. Structure and cytopathic effects of Nelson Bay virus. *Journal of Virology.* 6(1):100–106.

Gu SH, Lim BK, Kadjo B, Arai S, Kim J-A, Nicolas V, Lalis A, Denys C, Cook JA, Dominguez SR, Holmes KV, Urushadze L, Sidamonidze K, Putkaradze D, Kuzmin IV, Kosoy MY, Song J-W, Yanagihara R. 2014. Molecular phylogeny of hantaviruses harbored by insectivorous bats in Côte d'Ivoire and Vietnam. *Viruses.* 6:1897–1910.

Guo WP, Lin XD, Wang W, Tian JH, Cong ML, Zhang H-L, Wang M-R, Zhou R-H, Wang J-B, Li M-H, Xu J, Holmes EC, Zhang Y-Z. 2013. Phylogeny and origins of hantaviruses harbored by bats, insectivores, and rodents. *PLoS Pathology.* 9(2):e1003159.

Herbold, JR, Heuschele WP, Berry RL, Parsons MA. 1983. Reservoir of St. Louis encephalitis virus in Ohio bats. *Journal of American Veterinary Research*. 44:1889–1893.

Holbrook MR. 2012. Kyasanur Forest disease. *Antiviral Research.* 96(3):353–362.

Hu B, Chmura AA, Li J, Zhu G, Desmond JS, Zhang Y, Zhang W, Epstein JH, Daszak P, Shi Z. 2014. Detection of diverse novel astroviruses from small mammals in China. *Journal of General Virology*. 95:2442–2449.

Kading RC, Kityo R, Nakayiki T, Ledermann J, Crabtree MB, Lutwama J, Miller BR. 2015. Detection of Entebbe bat virus after 54 years. *American Journal of Tropical Medicine and Hygiene.* 93(3):475–477.

Kalunda M, Mukwaya LJ, Mukuye A, Lulu M, Sekyalo E, Wright J, Casals J. 1986. Kasokero virus: a new human pathogen from bats (*Rousettus aegyptiacus*) in Uganda. *American Journal of Tropical Medicine and Hygiene.* 35:387–392.

Kemenesi G, Dallos B, Görföl T, Boldogh S, Estók P, Kurucz K, Oldal M, Németh V, Madai M, Bányai K, Jakab F. 2014. Novel European lineages of bat astroviruses identified in Hungary. *Acta Viologica.* 58:95–98.

Kemenesi G, Zhang D, Marton S, Dallos B, Görföl T, Estók P, Boldogh S, Kurucz K, Oldal M, Kutas A, Bányai K, Jakab F. 2015. Genetic characterization of a novel picornavirus detected in *Miniopterus schreibersii* bats. *Journal of General Virology.* 96:815–821.

Kim GR, Lee YT, Park CH. 1994. A new natural reservoir of hantavirus: isolation of hantaviruses from lung tissues of bats. *Archives of Virology.* 134(1–2):85–95.

Kohl C, Kurth A. 2015. Bat reoviruses. In: *Bats and Viruses: A New Frontier of Emerging Infectious Diseases*. L-F Wang and C Cowled (eds). Wiley Blackwell: Hoboken, NJ, pp. 203–215.

Kohl C, Lesnik R, Brinkmann A, Ebinger A, Radonic A, Nitsche A, Muhldorfer K, Wibbelt G, Kurth A. 2012. Isolation and characterization of three mammalian orthoreoviruses from European bats. *PLoS ONE.* 7:e43106.

Lau SKP, Woo PCY, Lai KKY, Huang Y, Yip CCY, Shek C-T, Lee P, Lam CSF, Chan K-H, Yuen K-Y. 2011. Complete genome analysis of three novel picornaviruses from diverse bat species. *Journal of Virology.* 85(170):8819–8828.

Lelli D, Moreno A, Steyer A, Naglič T, Chiapponi C, Prosperi A, Faccin F, Sozzi E, Lavazza A. 2015. Detection and characterization of a novel reassortant mammalian orthoreovirus in bats in Europe. *Viruses.* 7:5844–5854.

Li Q, Sun X, Li Z, Liu Y, Vavricka CR, Qi J, Gao JF. 2012. Structural and functional characterization of neuraminidase-like molecule N10 derived from bat influenza A virus. *Proceedings of the National Academy of Sciences USA*. 109(46):18897–18902.

Liu S, Li X, Chen Z, Chen Y, Zhang Q, Liao Y, Zhou J, Ke X, Ma L, Xiao J, Wu Y, Chen Z, Zhou J, Zheng X, Li J, Chen Q. 2013. Comparison of genomic and amino acid sequences of eight Japanese encephalitis virus isolates from bats. *Archives of Virology.* 158(12):2543–2552.

Lorusso A, Teodori L, Leone A, Marcacci M, Mangone I, Orsini M, Capobianco-Dondona A, Camma' C, Monaco F, Savini G. 2015. A new member of the *Pteropine Orthoreovirus* species isolated from fruit bats imported to Italy. *Infection, Genetics and Evolution.* 30:55–58.

L'vov DK, Easterday B, Hinshow W, Dandurov Iu, Arkhipov PN. 1979. Isolation of strains of the Hong Kong complex (H3N2) influenza virus from *Nyctalus noctula* bats in Kazakhstan. *Voprosy Virusologii.* 4:338–341.

L'vov DK, Tsyrkin YM, Karas FR, Timopheev EM, Gromashevski VL, Veselovskaya OV, Osipova NZ, Fomina KB, Grebenyuk YI. 1973. "Sokuluk" virus, a new group B arbovirus isolated from *Vespertilio pipistrellus* Schreber, 1775, bat in the Kirghiz S.S.R. *Archiv für die gesamte Virusforschung*. 41(3):170–174.

Machain-Williams C, López-Uribe M, Talavera-Aguilar L, Carrillo-Navarrete J, Vera-Escalante L, Puerto-Manzano F, Ulloa A, Farfán-Ale JA, Garcia-Rejon J, Blitvich BJ, Loroño-Pino AM. 2013. Serologic evidence of flavivirus infection in bats in the Yucatan Peninsula of Mexico. *Journal of Wildlife Diseases.* 49(3):684–689.

Marfin AA, Peteresen LR, Eidson M, Miller J, Hadler J, Farello C, Werner B, Campbell GL, Layton M, Smith P, Bresnitz E, Cartter M, Scaletta J, Obiri G, Bunning M, Craven RC, Roehrig JT, Julian KG, Hinten SR, Gubler DJ, ArboNET Cooperative Surveillance Group. 2001. Widespread West Nile virus activity, Eastern United States, 2000. *Emerging Infectious Diseases.* 7:730–735.

McLean RG, Trevino HA, Sather GE. 1979. Prevalence of selected zoonotic diseases in vertebrates from Haiti, 1972. *Journal of Wildlife Diseases.* 15(2):327–330.

Miura T, Toyokawa K, Allen R, Sulkin SE. 1970. Studies of arthropod-borne virus infections in Chiroptera. VII. Serologic evidence of natural Japanese B encephalitis virus infection in bats. *American Journal of Tropical Medicine and Hygiene*. 19:88–93.

Mourya DT, Yadav PD, Basu A, Shete A, Patil DY, Zawar D, Majumdar TD, Kokate P, Sarkale P, Raut CG, Jadhav SM. 2014. Malsoor virus, a novel bat phlebovirus, is closely related to severe fever with thrombocytopenia syndrome virus and Heartland virus. *Journal of Virology.* 88(6):3605–3609.

Neill WA. 1985. Kaeng Khoi. In: *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*, 3rd edition. N. Karabatsos (ed.). American Society for Tropical Medicine and Hygiene: San Antonio, TX, pp. 533–534.

O'Connor J, Rowan L, Lawrence J. 1955. Relationships between the flying fox (genus *Pteropus*) and arthropod-borne fevers of North Queensland. *Nature*.176:472.

Osborne JC, Rupprecht CE, Olson JG, Ksiazek TG, Rollin PE, Niezgoda M, Goldsmith CS, An US, Nichol ST. 2003. Isolation of Kaeng Khoi virus from dead *Chaerephon plicata* bats in Cambodia. *Journal of General Virology.* 84(Pt 10):2685–2689.

Pavri KM, Singh KRP. 1965. Demonstration of antibodies against the virus of Kyasanur Forest disease (KFD) in the frugivorous bat, *Rousettus leschenaulti*, near Poona, India. *Indian Journal of Medical Research*. 5:956–961.

Pavri KM, Singh KR. 1968. Kyasanur forest disease virus infection in the frugivorous bat, *Cynopterus sphinx*. *Indian Journal of Medical Research.* 56(8):1202–1204.

Perea-Martínez L, Moreno-Sandoval HN, Moreno-Altamirano MM, Salas-Rojas M, García-Flores MM, Aréchiga-Ceballos N, Tordo N, Marianneau P, Aguilar-Setién A. 2013. Experimental infection of *Artibeus intermedius* bats with serotype-2 dengue virus. *Comparative and Immunological Microbiology and Infectious Diseases.* 36(2):193–198.

Pilipski JD, Pilipski LM, Riseley LS. 2004. West Nile Virus antibodies in bats from New Jersey and New York. *Journal of Wildlife Diseases.* 40:335–337.

Platt KB, Mangiafico JA, Rocha OJ, Zaldivar ME, Mora J, Trueba G, Rowley WA. 2000. Detection of dengue virus neutralizing antibodies in bats from Costa Rica and Ecuador. *Journal of Medical Entomology.* 37(6):965–967.

Poole DS, Yú S, Caì Y, Dinis JM, Müller MA, Jordan I, Friedrich TC, Kuhn JH, Mehle A. 2014. Influenza A virus polymerase is a site for adaptive changes during experimental evolution in bat cells. *Journal of Virology.* 88(21):12572–12585.

Price JL. 1978a. Isolation of Rio Bravo and a hitherto undescribed agent, Tamana bat virus, from insectivorous bats in Trinidad, with serological evidence of infection in bats and man. *American Journal of Tropical Medicine and Hygiene.* 27(1 Pt 1):153–161.

Price JL. 1978b. Serological evidence of infection of Tacaribe virus and arboviruses in Trinadadian bats. *American Journal of Tropical Medicine and Hygiene.* 27(1):162–167.

Pritchard LI, Chua KB, Cummins D, Hyatt A, Crameri G, Eaton BT, Wang L-F. 2006. Pulau virus: a new member of the *Nelson Bay orthoreovirus* species isolated from fruit bats in Malaysia. *Archives of Virology.* 151: 229–239.

Quan PL, Firth C, Conte JM, Williams SH, Zambrana-Torrelio CM, Anthony SJ, Ellison JA, Gilbert AT, Kuzmin IV, Niezgoda M, Osinubi MO, Recuenco S, Markotter W, Breiman RF, Kalemba L, Malekani J, Lindblade KA, Rostal MK, Ojeda-Flores R, Suzan G, Davis LB, Blau DM, Ogunkoya AB, Alvarez Castillo DA, Moran D, Ngam S, Akaibe D, Agwanda B, Briese T, Epstein JH, Daszak P, Rupprecht CE, Holmes EC, Lipkin WI. 2013. Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proceedings of the National Academy of Sciences USA*. 110(20):8194–8199.

Rajagopalan PK, Paul SD, Sreenivasan MA. 1969. Isolation of Kyasanur Forest disease virus from the insectivorous bat, *Rhinolophus rouxi* and from *Ornithodoros* ticks. *Indian Journal of Medical Research.* 57(5):805–808.

Sabino-Santos G Jr, Maia FG, Vieira TM, de Lara Muylaert R, Lima SM, Gonçalves CB, Barroso PD, Melo MN, Jonsson CB, Goodin D, Salazar-Bravo J, Figueiredo LT. 2015. Evidence of hantavirus infection among bats in Brazil. *American Journal of Tropical Medicine and Hygiene.* 93(2):404–406.

Sandekian V, Lim D, Prud'homme P, Lemay G. 2013. Transient high level mammalian reovirus replication in a bat epithelial cell line occurs without cytopathic effect. *Virus Research*. 173:327–335.

Seymour C, Dickerman RW. 1978. Venezuelan encephalitis virus infection in neotropical bats. III. Experimental studies on virus excretion and non-arthropod transmission. *American Journal of Tropical Medicine and Hygiene*. 27(2):307–312.

Seymour C, Dickerman RW, Martin MS. 1978a. Venezuelan encephalitis virus infection in neotropical bats. I. Natural infection in a Guatemalan enzootic focus. *American Journal of Tropical Medicine and Hygiene*. 27(2 Pt 1):290–296.

Seymour C, Dickerman RW, Martin MS. 1978b.Venezuelan encephalitis virus infection in neotropical bats. II. Experimental infections. *American Journal of Tropical Medicine and Hygiene*. 27(2 Pt 1):297–306.

Sotomayor-Bonilla J, Chaves A, Rico-Chávez O, Rostal MK, Ojeda-Flores R, Salas-Rojas M, Aguilar-Setien Á, Ibáñez-Bernal S, Barbachano-Guerrero A, Gutiérrez-Espeleta G, Aguilar-Faisal JL, Aguirre AA, Daszak P, Suzán G. 2014. Dengue virus in bats from southeastern Mexico. *American Journal of Tropical Medicine and Hygiene.* 91(1):129–131.

Steyer A, Gutiérrez-Aguire I, Kolenc M, Koren S, Kutnjak D, Pokorn M, Poljšak-Prijatelj M, Rački N, Ravnikar M, Sagadin M, Steyer AF, Toplak N. 2013. High similarity of novel orthoreovirus detected in a child hospitalized with acute gastroenteritis to mammalian orthoreoviruses found in bats in Europe. *Journal of Clinical Microbiology.* 51(11):3818–3825.

Sulkin SE, Allen R, Miura T, Toyokawa K. 1970. Studies of arthropod-borne virus infections in *Chiroptera*. VI. Isolation of Japanese B encephalitis virus from naturally infected bats. *American Journal of Tropical Medicine and Hygiene*. 19(1):77–87.

Sumibcay L, Kadjo B, Gu SH, Kang HJ, Lim BK, Cook JA, Song J-W, Yanagihara R. 2012. Divergent lineage of a novel hantavirus in the banana pipistrelle (*Neoromicia nanus*) in Côte d'Ivoire. *Virology Journal.* 9:34.

Sun X, Shi Y, Lu X, He J, Gao F, Yan J, Qi J, Gao GF. 2013. Bat-derived influenza hemagglutinin h17 does not bind canonical avian or human receptors and most likely uses a unique entry mechanism. *Cell Reports.* 3:769–778.

Tajima S, Takasaki T, Matsuno S, Nakayama M, Kurane I. 2005. Genetic characterization of Yokose virus, a flavivirus isolated from the bat in Japan. *Virology.* 332:38–44.

Thalmann CM, Cummins DM, Yu M, Lunt R, Pritchard LI, Hansson E, Crameri S, Hyatt X, Wang L-F. 2010. Broome virus, a new fusogenic Orthoreovirus species isolated from an Australian fruit bat. *Virology*. 402: 26–40.

Thompson NN, Auguste AJ, da Rosa APAT. 2015. Seroepidemiology of selected alphaviruses and flaviviruses in bats in Trinidad. *Zoonoses and Public Health*. 62:53–60.

Tong S, Li YU, Rivailler P, Conrardy C, Castillo DAA, Chen L-M, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle SA, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe KA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO. 2012. A distinct lineage of influenza A virus from bats. *Proceedings of the National Academy of Sciences USA*. 109(11):4269–4274.

Tong S, Zhu X, Li Y, Shi M, Zhang J, Bourgeois M, Yang H, Chen X, Recuenco S, Gomez J, Chen L-M, Johnson A, Tao Y, Dreyfus C, Yu W, McBride R, Carney PJ, Gilbert AJ, Chang J, Guo Z, Davis CY, Paulson JC, Stevens J, Rupprecht C, Holmes EC, Wilson IA, Donis RO. 2013. New World bats harbor diverse influenza A viruses. *PLoS Pathogens.* 9(10): e1003657.

Turkington HL, Juozapaitis M, Kerry PS, Aydillo T, Ayllon J, García-Sastre A, Schwemmle M, Hale BG. 2015. Novel bat influenza virus NS1 proteins bind double-stranded RNA and antagonize host innate immunity. *Journal of Virology.* 89(20):10696–10701.

Ubico SR, McLean RG. 1995. Serological study of neotropical bats in Guatemala for virus antibodies. *Journal of Wildlife Diseases*. 31(1):1–9.

van den Hurk AF, Smith CS, Field HE, Smith IL, Northill JA, Taylor CT, Jansen CC, Smith GA, Mackenzie JS. 2009. Transmission of Japanese encephalitis virus from the black flying fox, *Pteropus alecto*, to *Culex annulirostris* mosquitoes, despite the absence of detectable viremia. *American Journal of Tropical Medicine and Hygiene*. 81:457–462.

Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. 2015. Genomic characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses naturally infecting bats, shrews, and ticks. *American Journal of Tropical Medicine and Hygiene.* 93(5):1041–1051.

Wang J, Moore NE, Murray ZL, McInnes K, White DJ, Tompkins DM, Hall RJ. 2015. Discovery of novel virus sequences in an isolated and threatened bat species, the New Zealand lesser short-tailed bat (*Mystacina tuberculata*). *Journal of General Virology.* 96(8):2442–2452.

Wang JL, Pan XL, Zhang HL, Fu SH, Wang HY, Tang Q, Wang LF, Liang GD. 2009. Japanese encephalitis viruses from bats in Yunnan, China. *Emerging Infectious Diseases.* 15(6):939–942.

Wang L, Fu S, Cao L, Lei W, Cao Y, Song J, Tang Q, Zhang H, Feng Y, Yang W, Liang G. 2015. Isolation and identification of a natural reassortant mammalian orthoreovirus from least horseshoe bat in China. *PLoS ONE.* 10(3):e0118598.

Watanabe S, Omatsu T, Miranda MEG, Masangkay JS, Ueda N, Endo M, Kato K, Tohya Y, Yoshikawa Y, Akash H. 2010. Epizootology and experimental infection of Yokose virus in bats. *Comparative Immunology and Microbiology of Infectious Diseases.* 33:25–36.

Weiss S, Witkowski PT, Auste B, Nowak K, Weber N, Fahr J, Mombouli JV, Wolfe ND, Drexler JF, Drosten C, Klempa B, Leendertz FH, Kruger DH. 2012. Hantavirus in bat, Sierra Leone. *Emerging Infectious Diseases.* 18(1):159–161.

Xiao J, Li J, Hu G, Chen Z, Wu Y, Chen Y, Chen Z, Liao Y, Zhou J, Ke X, Ma L, Liu S, Zhou J, Dai Y, Chen H, Yu S, Chen Q. 2011. Isolation and phylogenetic characterization of bat astroviruses in southern China. *Archives of Virology.* 156:1415–1423.

Xu L, Wu J, He B, Qin S, Xia L, Qin M, Li N, Tu C. 2015. Novel hantavirus identified in black-bearded tomb bats, China. *Infection and Genetic Evolution*. 31:158–160.

Yamanaka A, Iwakiri A, Yoshikawa T, Sakai K, Singh H, Himeji D, Kikuchi I, Ueda A, Yamamoto S, Miura M, Shioyama Y, Kawano K, Nagaishi T, Saito M, Minomo M, Iwamoto N, Hidaka Y, Sohma H, Kobayashi T, Kanai Y, Kawagishi T, Nagata N, Fukushi S, Mizutani T, Tani H,Taniguchi S, Fukuma A, Shimojima M, Kurane I, Kageyama T, Odagiri T, Saijo M, Morikawa S. 2014. Imported case of acute respiratory tract infection associated with a member of species Nelson Bay orthoreovirus. *PLoS ONE.* 9(3):e92777.

Zhang Y-Z. 2014. Discovery of hantaviruses in bats and insectivores and the evolution of the genus Hantavirus. *Virus Research.* 187:15–21.

Zhu HC, Chu DKW, Liu W, Dong BQ, Zhang SY, Zhang JX, Li LF, Vijaykrishna D, Smith GJD, Chen HL, Poon LLM, Peiris JSM, Guan Y. 2009. Detection of diverse astroviruses from bats in China. *Journal of General Virology.* 90:883–887.

Zhu X, Yang H, Guo Z, Yu W, Carney PJ, Li Y, Chen L-M, Paulson JC, Donis RO, Tong S, Stevens J, Wilson IA. 2012. Crystal structures of two subtype N10 neuraminidase-like proteins from bat influenza A viruses reveal a diverged putative active site. *Proceedings of the National Academy of Sciences USA*. 109(46):18903–18908.

Zhu X, Yu W, McBride R, Li Y, Chen L-M, Donis RU, Tong S, Paulson JC, Wilson IA. 2013. Hemagglutinin homologue from H17N10 bat influenza virus exhibits divergent receptor-binding and pH-dependent fusion activities. *Proceedings of the National Academy of Sciences USA*. 110(4):1458–1463.

7

BALTIMORE CLASS I AND CLASS II DNA VIRUSES OF BATS

7.1 INTRODUCTION TO DOUBLE- AND SINGLE- STRANDED DNA VIRUSES

Baltimore Class I and Class II viruses utilize double-stranded and single-stranded DNA as their genetic information, respectively, and use a DNA-dependent DNA polymerase during replication and a DNA-dependent RNA-polymerase during transcription. These viruses have a wide range of sizes and appearances. Some of them are pathogenic, including the highly pathogenic poxviruses in which the resulting diseases may be disfiguring or have a high fatality rate. Other members of this group are defective and depend on the presence of a helper virus in order to replicate. See Table 7.1 for a list of Class I and Class II viruses with reported association to bats.

7.2 BALTIMORE CLASS I VIRUSES

Members of this viral class utilize double-stranded DNA as their genetic information. Class I viruses include poxviruses, adenoviruses, herpesviruses, papillomaviruses, and polyomaviruses.

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 7.1 Baltimore Class I and Class II viruses associated with bats

Bat family	Bat common name	Bat species	Virus
Pteropodidae	Sulawesi fruit bat	*Acerodon celebensis*	Bat polyomavirus 5b-2
Pteropodidae	Sulawesi fruit bat	*Acerodon celebensis*	Bat polyomavirus 6a
Vespertilionidae	Pallid bat	*Antrozous pallidus*	Adenovirus sp.
Vespertilionidae	Pallid bat	*Antrozous pallidus*	Cyclovirus GF-4c
Vespertilionidae	Pallid bat	*Antrozous pallidus*	Dependovirus
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	*Artibeus jamaicensis* bat parvovirus 1
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	PARV4-like virus, Aj-BtPV-1
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	PARV4-like virus, Al-BtPV-1
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	Polyomavirus R504, A1055, R104
Rhinolophoidea	Heart-nosed bat	*Cardioderma cor*	*Cardioderma* polyomavirus
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	Polyomavirus C1109
Phyllostomidae	Gray short-tailed bat	*Carollia subrufa*	Agua Preta virus
Molossidae	Free-tailed bats	*Chaerephon* sp.	Adenovirus sp.
Molossidae	Free-tailed bats	*Chaerephon* sp.	*Chaerephon* polyomavirus 1
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	Gammaherpesvirus sp.
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx*	Gammaherpesvirus sp.
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx*	Adenovirus sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Adenovirus sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	Polyomavirus AT7
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	Bat polyomavirus 5a
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	Bat polyomavirus 6b
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	Bat polyomavirus 6c
Pteropodidae	Malagasy fruit bats	*Eidolon dupreanum*	Alphaherpesvirus, Simplex virus genus
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Eidolon helvum* adenovirus 1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Beta-, gamma-, and alphaherpesvirus sp.
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Eidolon helvum* adenovirus 1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Eidolon helvum* bat parvovirus 1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Eidolon helvum* poxvirus 1

Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Eidolon* polyomavirus 1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	Papillomavirus EhelPV1
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	PARV4-like virus, Eh-BtPV-1
Vespertilionidae	Straw-colored fruit bat	*Emballonuroidea*	Gammaherpesvirus
Pteropodidae	Lesser dawn bat	*Eonycteris spelaea*	Betaherpesvirus
Vespertilionidae	Argentine brown bat	*Eptesicus furinalis*	Polyomavirus sp.
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Betaherpesvirus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Eptesipoxvirus
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	WA 2011
Vespertilionidae	Isabell's serotine	*Eptescicus isabellinus*	Papillomavirus EserPV2
Vespertilionidae	Isabell's serotine	*Eptescicus isabellinus*	Papillomavirus EserPV3
Vespteropodidae	Northern bat	*Eptesicus nilssonii*	Adenovirus sp.
Vespteropodidae	Serotine bat	*Eptesicus serotinus*	Bat gammaherpesvirus 1
Vespteropodidae	Serotine bat	*Eptesicus serotinus*	*Eptesicus serotinus* papillomavirus 1
Vespteropodidae	Serotine bat	*Eptesicus serotinus*	*Eptesicus serotinus* papillomavirus 2
Vespteropodidae	Serotine bat	*Eptesicus serotinus*	*Eptesicus serotinus* papillomavirus 3
Vespteropodidae	Serotine bat	*Eptesicus serotinus*	Adenovirus sp.
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	Polyomavirus R95
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	Adeno-associated virus
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	Adenovirus sp.
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	Circovirus sp.
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	Dependovirus
Hipposideridae	Noack's roundleaf bat	*Hipposideros* cf. *ruber*	Hepadnavirus, roundleaf bat hepatitis virus
Hipposideridae	Diadem leaf-nosed bat	*Hipposideros diadema*	*Hipposideros diadema* herpesvirus 1
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	Adeno-associated virus
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	Dependovirus
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	Gammaherpesvirus
Hipposideridae	Pomona roundleaf bat	*Hipposideros pomona*	Gammaherpesvirus
Vespertilionidae	Great evening bat	*Ia io*	Adenovirus AdV-4
Vespertilionidae	Great evening bat	*Ia io*	*Ia io* picornavirus 1

(*Continued*)

TABLE 7.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Phyllostomidae	Thomas's nectar bat	*Lonchophylla thomasi*	Alphaherpesvirus
Pteropodidae	Long-tongued nectar bat	*Macroglossus minimus*	Gammaherpesvirus sp.
Vespertilionidae	African long-fingered bat	*Miniopterus africanus*	Polyomavirus sp.
Miniopteridae	Eastern bent-winged bat	*Miniopterus fuliginosus*	Betaherpesvirus, BatBHV-2
Miniopteridae	Eastern bent-winged bat	*Miniopterus fuliginosus*	Gemycircularvirus
Miniopteridae	Greater long-fingered bat	*Miniopterus inflatus*	*Miniopterus* polyomavirus
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Adeno-associated virus
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Bat bufavirus
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Betaherpesvirus MsHV
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Circovirus sp.
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Dependovirus
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Gammaherpesvirus
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 1
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 2
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 3
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 4
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 5
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 6
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 7
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 8
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 9
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 10
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 11
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 12
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	*Miniopterus schreibersii* astrovirus 13
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersi*	*Miniopterus schreibersii* picornavirus 1
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Papillomavirus MschPV1
Miniopteridae	Common bent-winged bat	*Miniopterus schreibersii*	Papillomavirus MschPV2

Miniopteridae	Common bent-winged bat	*Miniopterus schreibersi*	Poxvirus
Molossidae	Pallas's mastiff bat	*Molossus molossus*	Cyclovirus sp.
Molossidae	Pallas's mastiff bat	*Molossus molossus*	Bat polyomavirus 3b
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	Cyclovirus sp.
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	Adenovirus sp.
Vespertilionidae	Brandt's bat	*Myotis brandtii*	Adenovirus sp.
Vespertilionidae	California myotis	*Myotis californicus*	Polyomavirus sp.
Vespertilionidae	Pond bat	*Myotis dasycneme*	Adenovirus sp.
Vespertilionidae	Daubenton's myotis	*Myotis daubentoni*	Adeno-associated virus
Vespertilionidae	Daubenton's myotis	*Myotis daubentoni*	Dependovirus
Vespertilionidae	Geoffroy's bat	*Myotis emarginatus*	Adenovirus sp.
Vespertilionidae	Horsfield's bat	*Myotis horsfieldii*	Adenovirus sp.
Vespertilionidae	Little brown bat	*Myotis lucifugus*	Cytomegalovirus sp.
Vespertilionidae	Little brown bat	*Myotis lucifugus*	Polyomavirus MyPyV
Vespertilionidae	Greater mouse eared bat	*Myotis myotis*	Adenovirus sp.
Vespertilionidae	Greater mouse eared bat	*Myotis myotis*	Bat gammaherpesvirus 2
Vespertilionidae	Greater mouse eared bat	*Myotis myotis*	Bat gammaherpesvirus 3
Vespertilionidae	Greater mouse eared bat	*Myotis myotis*	*Myotis myotis* bocavirus 1
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Adenovirus sp.
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bat gammaherpesvirus 1
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bat gammaherpesvirus 2
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bat gammaherpesvirus 3
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bat gammaherpesvirus 4
Vespertilionidae	Natterer's bat	*Myotis nattereri*	Bat betaherpesvirus 1
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Adeno-associated virus
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Bat adenovirus-3, strain TJM
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Circovirus sp.
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Dependovirus
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Gammaherpesvirus MrGHV-1
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Gammaherpesvirus MrGHV-2

(*Continued*)

TABLE 7.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	*Myotis ricketii* astrovirus 1
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	Papillomavirus MrPV1
Vespertilionidae	Rickett's big-footed bat	*Myotis* sp.	Circovirus sp.
Vespertilionidae	Lesser noctule	*Nyctalus leisleri*	Adenovirus sp.
Vespertilionidae	Common noctule	*Nyctalus noctula*	Adenovirus sp.
Vespertilionidae	Common noctule	*Nyctalus noctula*	Bat gammaherpesvirus 3
Vespertilionidae	Common noctule	*Nyctalus noctula*	Bat gammaherpesvirus 4
Vespertilionidae	Common noctule	*Nyctalus noctula*	Adenovirus, mastadenovirus group
Molossidae	Large-eared free-tailed bat	*Otomops martiensseni*	Adenovirus sp.
Molossidae	Large-eared free-tailed bat	*Otomops martiensseni*	*Otomops* polyomavirus 1
Molossidae	Large-eared free-tailed bat	*Otomops martiensseni*	*Otomops* polyomavirus 2
Vespertilionidae	Kuhl's pipistrelle	*Pipistrellus kuhlii*	Adenovirus sp.
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	Bat gammaherpesvirus 1
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	Bat gammaherpesvirus 5
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	Adenovirus sp.
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bat adenovirus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bat gammaherpesvirus 1
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bat gammaherpesvirus 6
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bat gammaherpesvirus 7
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bat betaherpesvirus 1
Vespertilionidae	Soprano pipistrelle	*Pipistrellus pygmaeus*	Adenovirus sp.
Vespertilionidae	Pipistrelles	*Pipistrellus* sp.	Circoviruses sp.
Phyllostomidae	Short-headed broad-nosed bat	*Platyrrhinus brachycephalus*	Polyomvirus sp.
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	Adenovirus sp.
Vespertilionidae	Brown long eared bat	*Plecotus auritus*	Bat gammaherpesvirus 7
Vespertilionidae	Brown long eared bat	*Plecotus auritus*	Circovirus sp.
Vespertilionidae	Brown long eared bat	*Plecotus auritus*	Cyclovirus sp.
Pteropodidae	Greater musky fruit bat	*Ptenochirus jagori*	Gammaherpesvirus sp.
Pteropodidae	Davy's naked-backed bat	*Pteronotus davyi*	*Pteronotus* polyomavirus

Pteropodidae	Parnell's mustached bat	*Pteronotus parnelli*	Polyomavirus R266
Pteropodidae	Ryukyu flying fox	*Pteropus dasymallus yayeyamae*	Ryukyu virus 1
Pteropodidae	Indian flying fox	*Pteropus giganteus*	Adenovirus sp.
Pteropodidae	Indian flying fox	*Pteropus giganteus*	Papillomavirus PgigPV
Pteropodidae	Lyle's flying fox	*Pteropus lylei*	Alphaherpesvirus, Simplexvirus genus
Pteropodidae	Pacific flying fox	*Pteropus tonganus*	Pacific flying fox-associated cycloviruses 1–3
Pteropodidae	Pacific flying fox	*Pteropus tonganus*	Pacific flying fox feces-associated gemycircularviruses 1–14
Pteropodidae	Pacific flying fox	*Pteropus tonganus*	Pacific flying fox feces-associated circular viruses 1–15
Pteropodidae	Pacific flying fox	*Pteropus tonganus*	Pacific flying fox associated multicomponent virus 1
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	Bat polyomavirus 5b-1
Pteropodidae	Flying foxes	*Pteropus* sp.	Bat polyomavirus 6d-1
Pteropodidae	Flying foxes	*Pteropus* sp.	Bat polyomavirus 6d-2
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Adeno-associated virus
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Bat coronavirus
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Circovirus sp.
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Dependovirus
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* foamy virus 1
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* pestivirus 1
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* picornavirus 1
Rhinolophidae	Halcyon horseshoe bat	*Rhinolophus alcyone*	Hepadnavirus, horseshoe bat Hepatitis B virus
Rhinolophidae	Mediterranean horseshoe bat	*Rhinolophus euryale*	Adenovirus sp.
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Adenovirus sp.
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Circovirus RfCV-1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Cyclovirus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Gemycircularvirus

(*Continued*)

TABLE 7.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Papillomavirus RferPV 1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	*Rhinolophus ferrumequinum* betaherpesvirus 1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	*Rhinolophus ferrumequinum* papillomavirus 1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Adenovirus, mastadenovirus group
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Adenovirus sp.
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Gemycircularvirus
Rhinolophidae	Wooly horseshoe bat	*Rhinolophus luctus*	Circovirus sp.
Rhinolophidae	Great-eared horseshoe bat	*Rhinolophus macrotis*	Adeno-associated virus
Rhinolophidae	Great-eared horseshoe bat	*Rhinolophus macrotis*	Dependovirus
Rhinolophidae	Pearson's horseshoe bat	*Rhinolophus pearsoni*	Adeno-associated virus
Rhinolophidae	Pearson's horseshoe bat	*Rhinolophus pearsoni*	Dependovirus
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus blythi*	Circovirus sp.
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus blythi*	Cyclovirus sp.
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus blythi*	Adenovirus sp
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus blythi*	Gammaherpesvirus sp.
Rhinolophidae	Large rufous horseshoe bat	*Rhinolophus rufus*	Gammaherpesvirus sp.
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Adeno-associated virus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Circovirus sp.
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Dependovirus
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	Rhinolophus sinicus astrovirus 1
Pteropodidae	Egyptian fruit bat	*Rousettus aegyptiacus*	Papillomavirus RaPV1
Pteropodidae	Egyptian fruit bat	*Rousettus aegyptiacus*	Betaherpesvirus sp.
Pteropodidae	Egyptian fruit bat	*Rousettus aegyptiacus*	Gammaherpesvirus, *Rhadinovirus* genus
Pteropodidae	Egyptian fruit bat	*Rousettus aegyptiacus*	Polyomavirus sp.
Pteropodidae	Geoffroy's rousette	*Rousettus amplexicaudatus*	Gammaherpesvirus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Circovirus sp.
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	Mastadenovirus sp.
Vespertilionidae	Lesser Asiatic yellow bat	*Scotophilus kuhlii*	Adeno-associated virus

Vespertilionidae	Lesser Asiatic yellow bat	*Scotophilus kuhlii*	Adenovirus sp.
Vespertilionidae	Lesser Asiatic yellow bat	*Scotophilus kuhlii*	Dependovirus
Vespertilionidae	Lesser Asiatic yellow bat	*Scotophilus kuhlii*	Gammaherpesvirus
Phyllostomidae	Little yellow-shouldered bat	*Sturnira lilium*	Polyomavirus B0454
Anelloviridae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Tadarida brasiliensis* circovirus 1
Anelloviridae	Brazilian free-tailed bat	*Tadarida brasiliensis*	Cyclovirus *Tadarida brasiliensis* (CyCV-TB)
Anelloviridae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Tadarida brasiliensis* polyomavirus 1
Anelloviridae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Tadarida brasiliensis* polyomavirus 2
Anelloviridae	Brazilian free-tailed bat	*Tadarida brasiliensis*	Torque teno adenovirus
Emballonuroidea	Black-bearded tomb bat	*Taphozous melanopogon*	Adenovirus sp.
Vespertilionidae	Lesser club-footed bat	*Tylonycteris pachypus*	Cyclovirus
Vespertilionidae	Greater bamboo bat	*Tylonycteris robustula*	Betaherpesvirus TrBHV-1
Vespertilionidae	Greater bamboo bat	*Tylonycteris robustula*	Tylonycteris robustula astrovirus 1
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	Hepadnavirus, tent-making virus hepatitis B virus
Vespertilionidae	Parti-colored bat	*Vespertilio murinus*	Adenovirus sp.
Vespertilionidae	Asian parti-colored bat	*Vespertilio superans*	Cyclovirus sp.

7.2.1 Poxviruses

Poxviruses are large (240 nm by 300 nm), brick-shaped, double-stranded DNA viruses which replicate in intracellular inclusions in the host cell's cytoplasm. These viruses are able to incorporate host genes into their own genome to escape host immune responses. They are also able to aid in the horizontal transfer of transposable elements between host species. Genetic engineering of relatively benign poxviruses to express genes from pathogenic viruses, such as genes encoding HIV surface proteins, have provided a means of constructing safer recombinant vaccines. Vaccinia and canarypox virus have been particularly useful in the production of such vaccines.

Most poxviruses are not beneficial to humans or animals, however. Smallpox, with its formerly wide-spread distribution and a form having an approximate fatality rate of 30%, was one of the most devastating infectious diseases of humans. Many of those who survived that illness bore deep, permanent, and disfiguring scars, often on the face. The elimination of all natural transmission of smallpox via well-executed use of vaccination ranks among medicine's greatest achievements and was made possible largely because of the strict human tropism of its causative agent, variola major, and to a slighter extent, the less pathogenic variola minor. Molluscum contagiosum virus is another poxvirus that results in a typically mild skin disease in humans characterized by lesions that usually resolves in 6–12 months without scarring.

Not all poxviruses are host-specific, however, and some poxvirus genera have broad host tropisms. These poxviruses have the potential to cause zoonotic diseases in humans, such as monkeypox, which causes a potentially fatal disease in humans that is similar to smallpox. Squirrels and prairie dogs as well as several species of Africa rodents are also infected by monkeypox. Cats are major reservoirs for cowpox, a poxvirus that results in nonscarifying lesions in humans. Due to the demonstrated ability of some poxviruses to infect several hosts, it is important to search for the existence of poxviruses in other animal species, including bats, in order to discern whether they can infect and cause disease in humans. Most poxviruses are able to enter a wide variety of host cell types. Their survival and replication in these cells are restricted by host features, including the lack of necessary host factors or the host's innate immune system (reviewed by Baker *et al*. 2013). Changes in poxvirus host range are usually due to gene duplication, gain, or loss rather than point mutations and tend to alter the host's anti-viral innate immune responses. Fifteen such genes have been identified (reviewed by Baker *et al*. 2013).

Several bat poxviruses are known to exist. *Eidolon helvum* poxvirus 1 and Eptesipox virus display very different characteristics and geographical ranges (reviewed by Baker & Murcia 2014). The first of these was detected in 13% ($n=40$) of healthy *E. helvum* in West Africa. *E. helvum* also hosts a virus that is similar to the molluscum contagiosum virus of humans. A bat poxvirus was found in the presence of nematodes in epidermal nodules in *Miniopterus schreibersii* in Australia and another bat poxvirus was found in the US in several diseased big brown bats (*Eptesicus fuscus*). The latter poxvirus was detected by PCR in the bats' wings and joints. Infected animals had necrosuppurative osteomyelitis in multiple joints (Emerson *et al*. 2013). In the past, a very small number of smallpox patients developed similar conditions of osteomyelitis with arthritis (osteomyelitis variolosa). Vaccinia osteomyelitis sometimes was seen in those vaccinated with vaccinia. Comparison of the bat poxvirus's DNA to that of previously discovered poxviruses suggests that it be placed in a new genus and named Eptesipox virus (Emerson *et al*. 2013).

7.2.2 Adenoviruses

Adenoviruses are nonenveloped, icosahedral viruses with linear, nonsegmented dsDNA and range in size from 70 to 100 nm. The family contains five genera: *Mastadenovirus* (in mammals), *Aviadenovirus, Atadenovirus, Ichtadenovirus*, and *Siadenovirus*. Adenoviruses infect all groups of vertebrates, with 52 known human serotypes. They are very common in humans and cause a range of infections that include respiratory disease, conjunctivitis, and gastroenteritis (reviewed by Y. Li *et al*. 2010b).

In Asia, primary spleen cell cultures from a healthy Ryukyu flying fox (*Pteropus dasymallus yayeyamae*), a Japanese fruit bat, were found to carry a *Mastadenovirus*, Ryukyu virus 1 (Maeda *et al*. 2008). Another *Mastadenovirus* was isolated from the fruit bat *Rousettus leschenaultii* in India (Raut *et al*. 2012). Several studies in southern China detected adenovirus DNA in eight bat species: *Hipposideros armiger, Myotis horsfieldii, Myotis ricketti, M. schreibersii, Scotophilus kuhlii, Taphozous melanopogon, Rhinolophus blythi,* and *Cynopterus sphinx* (Y. Li *et al*. 2010b; Zheng *et al*. 2016). They appear to be most prevalent in *Myotis* species and *S. kuhlii*. *C. sphinx*, however, harbored eight novel adenoviruses, with 13.3% of the samples testing positive in one of the studies (Zheng *et al*. 2016). Importantly, phylogenetic analysis showed a low similarity (57.1–69.3%) between human and bat adenoviruses, suggesting a lack of zoonotic infection. Many of the bat adenoviruses were instead most closely related to canine adenoviruses. AdV-TJM and AdV-4 were isolated from *M. ricketti* and the great evening bat *(Ia io)* from China (Y. Li *et al*. 2010b; Chen *et al*. 2012). Most animal and human cell lines are susceptible to infection *in vitro* that leads to cytopathic effect.

In Africa, metagenomic analysis revealed a novel species of adenovirus (*Eidolon helvum* adenovirus 1) in the throat and urine of the straw-fruit bat (*E. helvum*), a migratory bat species that is widely distributed throughout the continent. It lives in close proximity to humans and is used as bushmeat. The bat adenovirus was related to adenoviruses from humans, having 77–90% amino acid identity with one human virus protein (Baker *et al*. 2013). It should be noted that even small changes in amino acids, as little as one amino acid, may have very large effects upon viruses' ability to bind to receptors on other potential host species, abrogating their ability to infect new hosts. Adenovirus was also detected in approximately 2% of bat fecal samples from Kenya ($n=217$) (Conrardy *et al*. 2014). Viral DNA was found in *Chaerephon* species and *Otomops martiensseni*, and some of the latter bat species were also co-infected with either a paramyxovirus or polyomavirus.

In Europe, adenoviruses (AdV-2) were first isolated from bats in 2009 from deceased, previously moribund, common pipistrelles (Sonntag *et al*. 2009). High levels of virus were detected in the intestine and lower amounts in the liver and kidneys of these animals. Adenovirus DNA was later detected in 14.7% of 28 tested German and Hungarian bat species, primarily those belonging to the Vespertilionidae family ($n>300$). Of these, 28 adenovirus species were novel and six had been previously described (Vidovszky *et al*. 2015). More than one adenovirus species was present in some of the bat species, such as *N. noctula* (AdV-1, AdV-2, and AdV-3) and *P. pipistrellus* (AdV-2 and AdV-3). Additionally, some viruses infect more than one bat species, such as vespertilionid AdV-1 in *N. noctula, P. nathusii*, and a whiskered bat (Vidovszky *et al*. 2015).

In South America, adenovirus DNA was also detected by PCR in several Brazilian common vampire bats (*Desmodus rotundus*), demonstrating the wide range of bats carrying adenoviruses (Lima *et al*. 2013).

7.2.3 Herpesviruses

Herpesviruses are large, enveloped, dsDNA viruses that infect skin and mucosal membranes as well as the lymphatic and nervous systems of several vertebrate groups, including humans. After the initial infection is resolved, herpesviruses persist in a latent form in some cells and may be later reactivated, either symptomatically or asymptomatically, and be transmitted to another host, typically through close contact. The eight species of herpesviruses that infect humans are as follows: (a) varicella-zoster (causative agent of chickenpox and shingles), (b) Epstein-Barr virus (infectious mononucleosis; Burkitt's lymphoma), (c, d) human herpesviruses 6 and 7 (roseola infantum), (e) human herpesvirus type 8 (Kaposi sarcoma), (f) herpes simplex 1 (cold sores), (g) herpes simplex 2 (genital herpes), and (h) cytomegalovirus (mononucleosis; retinitis in immunocompromised people). While some of these herpesviruses cause mild skin lesions, others result in severe to fatal disease, including blindness and massive proliferation or cancers of human B lymphocytes, especially in immunocompromised hosts. Additionally, simian herpesvirus B, an alphaherpesvirus usually found in macaques, is able to cause severe zoonotic infection in humans, typically a fatal form of encephalitis in immunocompetent people.

Herpesviruses are divided in three subfamilies: Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. Gammaherpesviruses cause disease, including cancer, in many animal species. Interspecies transmission of gammaherpesviruses also occurs (Sano *et al*. 2015). Gammaherpesviruses are further divided into five subgroups, of which G2 and G3 are 'bat-only' subgroups. G1, G4, and G5 also contain prominent 'bat clades'. G1 also contains a 'bat-only' cluster of 33 herpesviruses composed of two groups of viruses: one in which the viruses are from *S. kuhlii*; and the other in which the majority of viruses are from *R. blythi*. Interestingly, bat members of the G5 subgroup are closely related to a bovine herpesvirus, sharing 97.0–98.5 % sequence identity, even though herpesviruses are often host-specific (reviewed by Zheng *et al*. 2015).

In Europe, DNA from seven novel gammaherpesviruses and one novel betaherpesvirus were discovered in seven different moribund or dead German Vespertilionidae family bats (Wibbelt *et al*. 2007). One half of these animals had pneumonia without pulmonary lesions. The sequences of six of the gammaherpesviruses were similar to the *Percavirus* and *Rhadinovirus* genera, while the seventh was more closely related to a porcine herpesvirus of the *Macavirus* genus. None of the above gammaherpesviruses were related to known human viruses. The bat betaherpesvirus, however, was distantly related to human cytomegalovirus. A study in Hungary demonstrated the presence of DNA of a new beta- and a new gammaherpesvirus in captive Egyptian fruit bats (*Rousettus aegyptiacus*) (Jánoska *et al*. 2011). A gammaherpesvirus from the *Rhadinovirus* genus was also detected in a serotine bat (*Eptesicus serotinus*) from Hungary by Molnár *et al*. (2008). The infected bat displayed jaundice and anorexia before death. The liver contained major lesions in addition to the virus.

Many herpesviruses are present in bats from tropical Africa, Madagascar, and Asia. Baker *et al*. (2013) used metagenomics analysis to identify 539 sequences related to

herpesviruses from *E. helvum* from Ghana, primarily from the bats' throat samples. Most of the positive samples were related to betaherpesviruses ($n=366$) or gammaherpesviruses ($n=171$), with only two sequences most closely related to alphaherpesviruses. Several alphaherpesvirus species were also found in throat swabs of a healthy Lyle's flying fox (*Pteropus lylei*) and Malagasy fruit bats (*Eidolon dupreanum*) from Cambodia and Madagascar, respeectively (Razafindratsimandresy *et al*. 2009).

In Japan, cells from 8% of tested *Miniopterus fuliginosus* ($n=50$) contained a betaherpesvirus, BatBHV-2, that caused cytopathic effect in primary kidney cell culture. BatBHV-2 was detected in the spleen, liver, kidneys, and lungs of the bats. This virus is most closely related to tupaiid herpesvirus 1(from tree shrews) and caviid herpesvirus 2 (guinea pig cytomegalovirus) (Watanabe *et al*. 2010). Another betaherpesvirus that is closely related to that of tree shrews was isolated and sequenced from *M. schreibersii* (MsHV) (Zhang *et al*. 2012). MsHV was discovered during preparation of primary culture bat lymph node cells as the cause of "spontaneous" cytopathic effect. This virus has several unique features. One of its predicted proteins is similar to a protein from the mildly pathogenic human herpesvirus 6. Several unique open reading frames (ORFs) appear to encode homologs of the following human immune response proteins: major histocompatibility complex (MHC)-related proteins, MHC class I and class II homologs, and homologs of C-type lectin- or natural killer cell lectin-like receptors. One of MsHV's proteins is a homolog of a HHV-7 molecule which downregulates classical and nonclassical MHC class I complexes from the cell surface. In addition, the MsHV genome encodes the predicted protein products of a unique viral gene family with sixteen members which have no significant sequence identity with other known proteins, however contain immunoglobulin-like beta-sandwich domains (Zhang *et al*. 2012). C-type lectin homologs of host cell proteins, including chemokine receptors, are also encoded by gammaherpesviruses and betaherpesviruses from other animal groups (reviewed in Zhang *et al*. 2012).

Two studies in the Philippines detected herpesviruses in bats. Watanabe *et al*. (2009) identified herpesvirus DNA from spleen tissue of *Hipposideros diadema*. This novel virus, tentatively named *Hipposideros diadema* herpesvirus 1, was most closely related to the gammaherpesviruses. Sano *et al*. (2015) also discovered DNA from a novel member of the gammaherpesvirus group in 20% of the intestinal ($n=70$) and 10% of lung ($n=69$) and blood clot samples ($n=52$). The percentage of infected bats from each of the gammaherpesvirus-positive species are as follows: 71% of *Ptenochirus jagori* ($n=7$), 28% of *Rousettus amplexicaudatus* ($n=40$), 25% of *Macroglossus minimus* ($n=4$), 16% of *Cynopterus brachyotis* ($n=19$), and 100% of *Rhinolophus rufus* ($n=1$).

A study of herpesvirus diversity in southern China detected viral DNA in 14.0% of fecal samples from apparently healthy bats ($n=520$) using nested PCR. Prevalence of herpesvirus DNA in various bat species were as follows: 6.7% of *Hipposideros larvatus* ($n=15$), 11% of *Hipposideros pomona* ($n=110$), 28.6% of *M. ricketti* ($n=7$), 3.5% of *M. schreibersii* ($n=144$), 20.9% of *S. kuhlii* ($n=177$), 33.3% of *Emballonuroidea* ($n=3$), 16.5% of *R. blythi* ($n=103$), and 15.0% of *C. sphinx* ($n=60$). Phylogenetic analysis found a high degree of molecular viral diversity in bats of different species and from different geographic regions, suggesting that co-evolution of bats and herpesviruses is occurring (Zheng *et al*. 2015). Even within a bat host, herpesviruses may be genetically diverse – viruses detected in *S. kuhlii* in the same geological region belonged to several Gammaherpesvirinae genera: *Percavirus, Lymphocryptovirus*, and *Macavirus*. Partial

amino acid pairwise similarities found within a single bat species ranged from 27.8 to 100%. Herpesviruses are present in bat digestive tracts and feces, suggesting that the oral–fecal route may be important in transmission (Zheng *et al*. 2015).

7.2.4 Papillomaviruses

Papillomaviridae are small, nonenveloped, dsDNA viruses that target the host's epithelium. They infect the skin and mucosa of mammals and also have been found in birds and reptiles. While most papillomavirus infections are asymptomatic, some human viruses are responsible for cancers, such as anogenital, head and neck, and skin cancers. Similarly, some bat papillomaviruses are benign, such as MschPV1 and MschPV2 from healthy *M. schreibersii* and MrPV1 from oropharyngeal or anal swabs from healthy *M. ricketti* (Tse *et al*. 2012; Wu *et al*. 2012). EhelPV1 was found in hair bulbs from a healthy *E. helvum* and partial sequencing revealed the presence of a novel papilloma virus in hair bulbs of an Indian flying fox (*Pteropus giganteus*). This virus was designated PgigPV1 (García-Pérez *et al*. 2013). Papillomaviruses are also linked to malignant lesions in animals, including basosquamous carcinoma on the wing of an Egyptian fruit bat (*R. aegyptiacus*), designated RaPV1 (Rector *et al*. 2006). Genomic sequences of another papilloma virus were also detected in *E. helvum* by Baker *et al*. (2013). This virus has 64% amino acid identity with a papilloma virus from *R. aegyptiacus* (*Rousettus aegyptiacus* papilloma virus 1).

A study of Iberian bats detected four novel papillomaviruses in mucosa of free-ranging *E. serotinus* (designated EserPV1, EserPV2, and EserPV3) and *Rhinolophus ferrumequinum* (RferPV1) (García-Pérez *et al*. 2014). Papillomaviruses are generally believed to be highly host-species specific, however EserPV2 and EserPV3 are able to infect both *E. serotinus* and *E. isabellinus*, suggesting a lack of strict host specificity.

7.2.5 Polyomaviruses

Polyomaviruses are small dsDNA viruses with a circular genome. They typically do not cause acute disease in immunocompetent hosts but rather are associated with subclinical infections and life-long persistence. Reactivation of a prior infection with the Merkel polyomavirus during a period of immunologic dysfunction, however, may lead to the highly aggressive Merkel cell skin cancer in humans. Similarly, reactivation of JC and BK polyomaviruses is linked to multifocal leukoencephalopathy and nephropathy in humans. The mouse polyomavirus also may cause lethal disease when inoculated into newborn mice and avian polyomaviruses frequently kill infected birds.

In the Americas, a study from northern Canada detected and/or sequenced polyomavirus DNA in 13% of healthy little brown bats (*Myotis lucifugus*) ($n = 31$), with a proposed name of *Myotis* polyomavirus (MyPyV). Polyomavirus DNA was also recovered from two California myotis (*Myotis californicus*) in the same study (Misra *et al*. 2009). DNA from two novel polyomaviruses, *Tadarida brasiliensis* polyomavirus 1 and 2 (TbPyV1 and TbPyV2) were detected by high-throughput sequencing in *Tadarida brasiliensis* bats from Brazil (de Sales Lima *et al*. 2015). These viruses are genetically distinct, sharing 69.8% whole-genome pairwise identity with each other and 74–78% nucleotide sequence identity to other bat polyomaviruses. Phylogenetic analysis of this

DNA clusters with DNA from polyomaviruses of *Otomops* and *Chaerephon* bats as well as DNA from viruses of some simians, including chimpanzees. It is possible that some polyomaviruses have transitioned between nonhuman primates and bats, but the direction of the transition is not known. It is unlikely that humans acquired polyomaviruses from bats since the known bat and human viruses are not closely related (de Sales Lima *et al*. 2015).

In South America's French Guiana, polyomaviruses were present in 13.5% of bat spleens ($n = 163$) from nine of twenty-two tested bat species (Fagrouch *et al*. 2012). The newly reported polyoma-positive bat species and their viruses are as follows: the flat-faced fruit-eating bat (*Artibeus planirostris* R504 – an A cluster virus; A1055 – B cluster; and R104 – C cluster), the common vampire bat (*D. rotundus* – A cluster), Pallas's mastiff bat (*Molossus molossus* B1130 – B cluster), Seba's short-tailed bat (*Carollia perspicillata* C1109 – C cluster), the Argentine brown bat (*Eptesicus furinalis*), Pallas's long-tongued bat (*Glossophaga soricina*), the short-headed broad-nosed bat (*Platyrrhinus brachycephalus*), Parnell's mustached bat (*Pteronotus parnelli* R266 – A cluster), and the little yellow-shouldered bat (*Sturnira lilium* B0454 – B cluster). *A. planirostris* R104 and *C. perspicillata* C1109 share some characteristics with the human Merkel cell polyomavirus, a gorilla polyomavirus, and two chimpanzee polyomaviruses. Interestingly, these species of nonhuman primates are not found in the New World

In Southeast Asia, a phylogenetic study of polyomavirus whole viral DNA in Indonesian fruit bats' spleens indicated the presence of two distinct genetic clusters which appear to belong with viruses from *Orthopolyomavirus* genus rodents (Kobayashi *et al*. 2015), as proposed by the International Committee on Taxonomy of Viruses. This viral group encompasses polyomaviruses from bats, primates, humans, cattle, and rodents. One of these clusters appears to be related to polyomaviruses of primates, including trichodysplasia spinulosa-associated polyomavirus in humans, a rare condition of cutaneous eruption of spiny papules, primarily on the face. Indonesian bat species from which polyomavirus DNA was isolated are: *Dobsonia moluccensis* (BatPyV5a – cluster D and BatPyV6a and BatPyV6b – cluster E), *Pteropus vampyrus* (BatPyV5b-1 – cluster D), and *Acerodon celebensis* (BatPyV5b-2 – cluster D and BatPyV6a – cluster E) (Kobayashi *et al*. 2015).

In Africa, Tao *et al*. (2013) conducted a study of polyomavirus diversity in predominantly healthy Kenyan and Guatemalan bats ($n = 195$, 22 bat species and $n = 96$, 13 bat species, respectively). Substantially more polyomaviruses were detected in the rectal or oral swabs from the Kenyan, than the Guatemalan, bats (11.8% versus 1.0%). The following bat species were found to harbor polyomaviruses: 31.6% of *O. martiensseni* ($n = 19$), 22.9% of *Chaerephon* species ($n = 35$), 22.2% of *E. helvum* ($n = 9$), 10.9% of *R. aegyptiacus* ($n = 46$), 7.1% of *Cardioderma cor* ($n = 14$), 100% of *Miniopterus africanus* ($n = 1$), and 5.9% of *Pteronotus davyi* ($n = 17$). The *P. davyi* was from Guatemala and the remainder of the animals from Kenya. Four of the polyomaviruses in this study are believed to be recombinants (Tao *et al*. 2013). This study provides evidence of great genetic diversity in bat-associated polyomavirus lineages since they were present in almost all of major polyomavirus clades with the exception of the avian clade. Since phylogenetic analysis indicate that these viruses formed a paraphyletic group, multiple transfers of polyomaviruses may have occurred among bats and other mammals, as suggested above for transfers to nonhuman primates.

7.3 BALTIMORE CLASS II VIRUSES

Members of this viral class utilize single-stranded DNA as their genetic information. Class II viruses include parvoviruses, dependoviruses such as adeno-associated viruses, circular replication-associated protein encoding single-stranded (CRESS) DNA viruses, and anelloviruses. As a class, these viruses are less pathogenic to humans than members of Class I DNA viruses. See Table 7.1 for a list of Class II (as well as Class I) viruses with reported association to bats.

7.3.1 Parvoviruses

The viral family Parvoviridae is composed of nonenveloped viruses with a 5 Kb ssDNA genome. Parvoviruses have the highest mutation rate of any DNA virus family, almost as high as is seen in RNA viruses. They are also extremely prone to recombination (reviewed by Kemenesi *et al*. 2015). The subfamily Parvovirinae contains eight genera of viruses that infect humans and other vertebrates. At least five groups of parvoviruses infect humans: parvovirus B19, human bocaviruses, PARV4-like viruses, bufaviruses, and dependoviruses. Parvoviruses have been linked to a wide range of acute and chronic illnesses in humans and animals (reviewed by Kemenesi *et al*. 2015). Parvovirus B19 causes fifth disease, which in children typically manifests as a mild rash. In adults, infection may lead to painful joints and severe anemia (CDC 2016). Human bocaviruses have been detected in stool or nasopharyngeal samples from children with gastroenteritis or severe acute respiratory tract infection (Song *et al*. 2010). In 2012, a bufavirus was discovered that infects humans and may cause acute diarrhea in children. It has been reported in Northern Europe and Bhutan, Southeast Asia (reviewed in Kemenesi *et al*. 2015). Dependoviruses include adeno-associated viruses 1 and 2. They are dependent upon the presence of a helper virus, such as an adenovirus, in order to be infectious. Infection with these viruses is asymptomatic in humans.

A 2011 study detected the genomic sequence of a PARV4-like virus, *Eidolon helvum* parvovirus 1 (Eh-BtPV-1), in frugivorous *E. helvum* bats from Ghana. This parvovirus has 41.4% amino acid identity with primate PARV4-like viruses in the largest viral protein, NS1. The study also discovered the first parvovirus member of a proposed new viral species and genus, *Artibeus jamaicensis* bat parvovirus 1 (Aj-BtPV-1), in the frugivorous *A. jamaicensis* bats and *A. lituratus* from Panama (Canuti *et al*. 2011). These *Eidolon* and *Artibeus* bat species differ greatly phylogenetically as well as in geological location. Viral prevalence in colonies among these bat species was 5.5–8%. Both of these bat parvoviruses are present in high concentrations in the blood: as high as 10^8 and 10^{10} copies/ml for Aj-BtPV-1 and Eh-BtPV-1, respectively. Eh-BtPV-1 was also found in all of the tested bat organs: brain, lungs, liver, spleen, kidneys, and intestine. Viral replication is believed to occur in the spleen and kidneys. Of note, since no other viruses were detected in the study, it appears that these viruses are able to replicate independently, unlike many members of this group (Canuti *et al*. 2011).

A novel parvovirus, a European bat bufavirus, was discovered during a metagenomic analysis of the insectivorous *M. schreibersii* fecal samples in Hungary (Kemenesi *et al*. 2015). The two bat bufavirus isolates had 64–77% nucleotide and 43–61% amino acid identity with seven different human bufavirus isolates. The novel bat bufavirus, therefore, appears to be somewhat related to human bufaviruses of the *Protoparvovirus*

genus, particularly the *Primate protoparvovirus* 1 species. Recombination analysis suggests that an intragenic recombination event occurred in a viral protein segment, thus the nonhuman primate bufavirus might have resulted from a past recombination between bat and human bufaviruses (Kemenesi *et al*. 2015).

Ten parvovirus genetic sequences were detected from the throat ($n=8$) and urine ($n=2$) of *E. helvum* (Baker *et al*. 2013). These are related to both members of the viral Parvoviridae subfamily found in mammals and from the Densovirinae subfamily, typically found in invertebrates (Baker *et al*. 2013). This virus is distinct from human parvoviruses but is related to members of the *Erythrovirus* and *Betaparvovirus* viral genera.

7.3.2 Dependoviruses

Adeno-associated viruses are members of the *Dependovirus* genus of parvoviruses and have a wide distribution in primates. Most adeno-associated viruses are only infectious in the presence of adenovirus or herpesvirus "helper viruses." A study of fecal swab samples from 19 bat species in China revealed adeno-associated viruses in 10 bat species with a mean prevalence rate of 22.4% ($n=83$) (Y. Li *et al*. 2010a). Species found to harbor adeno-associated viruses were*: Hipposidero armiger* (13.9%; $n=36$), *H. larvatus* (23.1%; $n=13$), *Minipopterus schreibersii* (30%; $n=10$), *Myotis daubentoni* (38.9%; $n=18$), *M. ricketti* (31.1%; $n=90$), *Rhinolophus affinis* (23.3%; $n=60$), *Rhinolophus macrotis* (25%; $n=4$), *Rhinolophus pearsoni* (10%: $n=10$), *Rhinolophus sinicus* (26.1%; $n=46$), and *S. kuhlii* (34.6%; $n=26$). Interestingly, all 27 fecal samples from *H. pomona* tested negative, however, two closely related species, *H. armiger* and *H. larvatus*, were positive (13.9% of 36 samples and 23.1% of 13 samples, respectively). It should be noted that less than 10 samples were assayed in seven of the nine bat species which tested negative.

Genetic analysis of the adeno-associated viruses' two large ORFs indicates that the bat adeno-associated viruses are relatively distantly related to known primate viruses and are phylogenetically closer to those from pigs. The bat adeno-associated viruses display remarkably large genetic diversity, having an average pairwise nucleotide identity of only 84.3 % (Y. Li *et al*. 2010a). These bat adeno-associated viruses may be divided into seven sublineages which lack host species specificity, but have a semi-restricted geographical distribution pattern.

7.3.3 Circular replication-associated protein encoding single-stranded DNA viruses

CRESS viruses are ubiquitous viruses that include the *Circoviridae, Geminiviridae*, and *Nanoviridae* families: only *Circoviridae* have been reported in bats. They all encode the replication-associated protein (Rep) essential for initiating rolling circle replication. Fecal material from infected hosts bear large numbers of diverse CRESS viruses and their DNA is utilized for viral species analysis.

The Circoviridae family contains the *Anellovirus, Circovirus*, and *Cyclovirus* genera. This family of CRESS viruses has ambisense, circular ssDNA with a size of about 2 kb, the smallest of the known autonomously replicating viral genomes (reviewed by Li *et al*. 2011). Their virions have nucleocapsids of approximately 20 nm in diameter and lack an envelope. The genome of circoviruses contains two characteristic major

ORFs that encode the replicase (Rep) and capsid (Cap) proteins that are arranged inversely. These viruses infect a wide range of vertebrates.

Anelloviruses include torque teno virus and torque teno mini virus. These are small, nonenveloped viruses with a circular negative ssDNA genome with no significant sequence homology with other known animal circoviruses. They infect several animal species, including humans, nonhuman primates, bats, cats, dogs, pigs, sea lions, and mosquitoes. Both of these viruses are highly prevalent in human bodily fluids and are spread via saliva droplets, the fecal–oral route, or breast milk (Biagini 2004). Healthy Brazilian free-tailed bats (*T. brasiliensis*) are infected with a member of the torque teno group (Cibulski *et al*. 2014). This bat virus shares 32% amino acid sequence identity with human torque teno mini virus 2.

Circovirus infections in humans are asymptomatic, however, circoviruses may cause diseases in other animal species. Infection of pigs with porcine circovirus 2 leads to poor growth rate, wasting, and systemic inflammatory lesions (Merck Veterinary Manual 2014). Circovirus infection in birds may lead to beak and feather disease, infectious anemia, and, in some bird species, death. In dogs, canine circoviruses have been linked to bloody vomiting and diarrhea (AVMA 2013).

A metagenomics analysis of respiratory fluid samples from Brazilian free-tailed bats (*T. brasiliensis*) led to the discovery of a novel bat circovirus, *Tadarida brasiliensis* circovirus 1 (TbCV-1). It shares a 75.4% acid identity with TbCV-1 and *R. ferrumequinum* circovirus 1 from Chinese bats (Lima 2015a). A separate study conducted in the Tongan archipelago of Oceania found three new species of cycloviruses and 14 new geminincircularviruses in the Pacific flying fox (*Pteropus tonganus*) fecal samples as well as 16 unclassified CRESS viruses, one of which has a multicomponent genome (Male *et al*. 2016). One of the new Pacific flying fox cycloviruses has a high similarity (about 77% genome-wide identity) with a human cyclovirus identified in a patient with paraplegia of unknown etiology in Malawi, Africa (Male *et al*. 2016). CRESS viruses are therefore present in bats from several continents.

A study of viral diversity in Chinese bats utilized inverse PCR analysis of bat fecal material. Full-length sequences of five novel bat circoviruses (YN-BtCV-1 to -5) were discovered in several bat species. The Rep protein sequences had amino acid identities of 51–72% with previously reported circoviruses and 25–69% identity among themselves, while the Cap protein sequences had amino acid identities of 7–56% with known circoviruses and 5–36% identity among themselves (Ge *et al*. 2011). These five viruses fell within the cyclovirus group (see below). The bat species from which the circovirus or cyclovirus DNA were found are the following: *Rousettus leschenaultia, Rhinolophus pusillus, Rhinolophus luctus, H. armiger, Myotis* sp., and *M. schreibersii*. The prevalence of circovirus-like genomes in the different bat species ranged from 2.6 to 66.7%. Of note, YN-BtCV-2 is related to the human cyclovirus PK5034 from Pakistan and YN-BtCV-5 is related to the human cyclovirus NG14 from Nigeria. Both of the human cycloviruses were found in stool samples.

Circoviridae DNA was detected in fecal pellets from a bat colony in southern Brazil (Lima *et al*. 2015b). The colony was estimated to harbor about 500 bat specimens of velvety free-tailed bats (*M. molossus*) and Brazilian free-tailed bats (*T. brasiliensis*). Whole-genome characterization of bat fecal material detected four new circular ssDNAs viruses from the family Circoviridae: two circoviruses and two cycloviruses. It is unclear which of the two bat species harbored these viruses.

Cycloviruses are very similar to circoviruses, but have several differences, including a smaller genome, smaller Rep and Cap proteins, lack of a 3' intergenic region, and a longer 5' intergenic region. Cycloviruses have been reported in cerebrospinal fluid from humans and are linked to human neurological and respiratory disease (Garigliany *et al*. 2014). The human Cyclovirus-Vietnam (CyCV-VN) has been found in fecal samples from pigs and humans in Southeast Asia and Africa, demonstrating a wide distribution for members of this viral genus and their ability to infect other mammals.

Samples of pectoral muscle, digestive tract, and fecal material of an adult *T. brasiliensis* in Texas, in the southern US, were PCR-positive for a circovirus-like gene. Full-length sequencing of the genome from muscle placed this virus within the cyclovirus clade, tentatively named cyclovirus *Tadarida brasiliensis* (CyCV-TB). Its Rep protein had 44–71% similarity to that of other cycloviruses and had the highest amino acid similarities to CyCV-NG12 from Nigerian human feces and the CyCV-GF4 genome from bat guano from California in the US (L. Li *et al*. 2010). The amino acid similarities of the CyCV-TB Cap protein were much lower: 12–48% similarity to cycloviruses of humans and chimpanzees and 28% to that of CyCV-GF4 (Li *et al*. 2011).

7.4 CONCLUSIONS

A great degree of diversity exists among double- and single-stranded DNA viruses in terms of size (the very small polyomaviruses to the huge poxviruses) and pathogenicity (ranging from asymptomatic, mild disease, to severe or fatal infections). Genetic diversity among some of these groups is also great, sometimes even within a viral species. Some of these viruses are defective and require the assistance of a helper virus in order to replicate. Many of the DNA viral species are highly host-specific while others infect a wider range of hosts.

The variola major poxvirus was the most common cause of smallpox, a devastating disease that ravaged human populations before its eradication from natural transmission. Variola was extremely host-specific, infecting only humans. Monkeypox virus, however, is somewhat less host-specific and is also able to infect humans and cause disease similar to smallpox. However, poxviruses of other animal species are proving to be extremely helpful in vaccine development as nonpathogenic carriers of exogenous genetic information. Such poxviruses, including canarypox virus, are being developed as carriers of HIV genes. Very few poxviruses have been reported in bat species from either the Old or New World. The *Eidon helvum* poxvirus 1 has been found in apparently healthy populations of the African bats. This bat virus has some similarities to molluscum contagiosum virus, which causes a mild skin disease in immunocompetent humans. Poxviruses have also been reported in *Eptesicus fuscus*, from the Americas, and the widely distributed Old World bat, *Miniopterus schreibersii*. The Eptesipox virus has been found to cause necrosuppurative osteomyelitis in the joints of bats.

Adenoviruses are found in virtually all major groups of vertebrates and 52 species are known to infect humans, some of these causing respiratory disease and gastroenteritis. Adenoviruses have been found in many species of apparently healthy bats from most continents of the world. While found in bats with very diverse dietary habits, the majority of bat adenoviruses are found in insectivorous bats, particularly from the family Vespertilionoidea. Adenoviruses are present in very few frugivorous bats, all from the

family Pteropodidae in Asia. Adenoviruses have been reported in one carnivorous bat species, *Noctilio leporinus*, and in the hematophagous common vampire bat of Latin America. At least one adenovirus is highly pathogenic to common pipistrelles in Europe.

Herpesviruses are highly diverse among bat species and in animals from different geographical regions, suggesting that co-evolution is occurring between these viruses and bats. While several frugivorous or nectivorous bat species, almost all from the family Pteropodidae, serve as hosts to herpesviruses, the vast majority of herpesvirus-infected bats are insectivores from the familes Vespertilionoidea, Rhinolophidae, or Miniopteridae. Many herpesviruses cause relatively mild disease, but some are responsible for severe to fatal disease in bats and humans. Gammaherpesviruses have been found in either moribund or dead European Vespertilionoidae bats. Some of the bats had pneumonia and others had liver disease. While herpesviruses are typically host-specific, simian herpesvirus B of macaques causes a fatal neurological zoonotic infection in humans. One European bat betaherpesvirus was found to be distantly related to human cytomegalovirus, a virus found in about 80% of the human population and which is responsible for blindness in some HIV-positive individuals. It is rarely pathogenic in immunocompetent people. A Japanese betaherpesvirus appears to be more closely related to cytomegaloviruses from tree shrews and guinea pigs.

Relatively few papillomaviruses infect Old World bats, with no reported infections of bats from the Americas. This group of viruses tends to be highly host-specific. Infection is usually asymptomatic but, in some cases, has been linked to skin malignancies on bat wings or cancer in humans.

Polyomaviruses are found in bats of all dietary habits. While found in both New and Old World bats, many of the bats hosting polyomaviruses are from the Americas, a few bat species from Africa or Oceania, and none from Europe or Asia. Polyomaviruses usually are not responsible for acute disease, but rather cause life-long, subclinical infection. If reactivated, however, several human polyomaviruses (Merkel cell polyomavirus and JC and BK polyomaviruses) may lead to severe or fatal human disease. Bat polyomaviruses are not closely related to those of humans, but some of these viruses cluster with Old World nonhuman primate viruses and two South American bat polyomaviruses have some characteristics of Merkel cell polyomavirus.

Several groups of ssDNA viruses infect bats or humans, many of these are defective viruses and require infection with a helper virus in order to replicate. They are found primarily in insectivorous bats of several genera, especially *Hipposideros, Rhinolophus, Myotis*, and *Miniopterus*. They are almost exclusively found in Old World bats, with the exception of *Antrozous pallidus* from North America and *Artibeus jamaicensis* and *Artibeus lituratus* from Latin America. Parvoviruses have a very high rate of mutation and are prone to recombination. They cause the mild fifth disease in humans. Infection with human bufaviruses and bocaviruses may also result in severe respiratory or gastrointestinal disease in children. A bat parvovirus was found to have a small degree of amino acid identity to one human PARV4 protein and two bat bufaviruses have a low degree of amino acid similarity to some human bufavirus isolates. Adeno-associated viruses demonstrate a wide range of diversity among bats. While dependoviruses in general are widely distributed among primates, those from bats are most closely related to viruses found in pigs.

CRESS viruses (circoviruses and cycloviruses) are ssDNA viruses present primarily in insectivorous bats. They are also found almost exclusively in Old World bats,

with the exception of *Tadarida brasiliensis* and *Antrozous pallidus*. They cause either asymptomatic infection in humans and bats (circoviruses) or respiratory and neurological diseases, as is sometimes the case for cycloviruses. A low degree of amino acid similarity exists between some cycloviruses of humans and bats.

Taken together, many of the DNA viruses that infect bats tend to be species-specific. While some of these viral groups have a wide geographical distribution, others have a much more restricted range. Most of the bats infected with DNA viruses are insectivores, many of them from the family Vespertilionoidae. While many of the above viruses cause, at the most, mild disease, some others are responsible for severe or fatal infections in bats, humans, and other vertebrates.

REFERENCES

AVMA. 2013. Circovirus in dogs FAQ. https://www.avma.org/KB/Resources/Pages/Circovirus-in-Dogs-Frequently-Asked-Questions.aspx. Accessed May 16, 2016.

Baker KS, Leggett RM, Bexfield NH, Alston M, Daly G, Todd S, Tachedjian M, Holmes CEG, Crameri S, Wang L-F, Heeney JL, Suu-Ire R, Kellam P, Cunningham AA, Wood JLN, Caccamo M, Murcia PR. 2013. Metagenomic study of the viruses of African straw-coloured fruit bats: detection of a chiropteran poxvirus and isolation of a novel adenovirus. *Virology.* 441:95–106.

Baker KS, Murcia PR. 2014. Poxviruses in bats … so what? *Viruses.* 6:1564–1577.

Biagini P. 2004. Human circoviruses. *Veterinary Microbiology.* 98(2):95–101.

Canuti M, Eis-Huebinger AM, Deijs M, de Vries M, Drexler JF, Oppong SK, Müller MA, Klose SM, Wellinghausen N, Cottontail VM, Kalko EKV, Drosten C, van der Hoek L. 2011. Two novel parvoviruses in frugivorous New and Old World bats. *PLoS ONE.* 6(12):e29140.

CDC. 2016. Parvovirus B19 and Fifth Disease. https://www.cdc.gov/parvovirusb19/fifth-disease.html. Accessed January 27, 2017.

Chen, LH, Wu ZQ, Hu YF, Yang F, Yang J, Jin Q. 2012. Genetic diversity of adenoviruses in bats of China. *Bing Du Xue Bao.* 28:403–408.

Cibulski SP, Teixeira TF, de Sales Lima FE, do Santos HF, Franco AC, Roehe PM. 2014. A novel Anelloviridae species detected in *Tadarida brasiliensis* bats: first sequence of a chiropteran Anellovirus. *Genome Announcements.* 2(5):e01028-14.

Conrardy C, Tao Y, Kuzmin IV, Niezgoda M, Agwanda B, Breiman RF, Anderson LJ, Rupprecht CE, Tong S. 2014. Molecular detection of adenoviruses, rhabdoviruses, and paramyxoviruses in bats from Kenya. *American Journal of Tropical Medicine and Hygiene.* 91(2):258–266.

de Sales Lima FE, Cibulski SP, Witt AA, Franco AC, Roehe PM. 2015. Genomic characterization of two novel polyomaviruses in Brazilian insectivorous bats. *Archives of Virology.* 160(7):1831–1836.

Emerson GL, Nordhausen R, Garner MM, Huckabee JR, Johnson S, Wohrle RD, Davidson WB, Wilkins K, Li Y, Doty JB, Gallardo-Romero NF, Metcalfe MG, Karem KL, Damon IK, Carroll DS. 2013. Novel poxvirus in big brown bats, Northwestern United States. *Emerging Infectious Diseases.* 19(6):1002–1004.

Fagrouch Z, Sarwari R, Lavergne A, Delaval M, de Thoisy B, Lacoste V, Verschoor EJ. 2012. Novel polyomaviruses in South American bats and their relationship to other members of the family *Polyomaviridae*. *Journal of General Virology.* 93:2652–2657.

García-Pérez R, Gottschling M, Wibbelt G, Bravo IG. 2013. Multiple evolutionary origins of bat papillomaviruses. *Veterinary Microbiology.* 165(1–2):51–60.

García-Pérez R, Ibáñez C, Godínez JM, Aréchiga N, Garin I, Pérez-Suaárez G, de Paz O, Juste J, Echevarrı JE, Bravo IG. 2014. Novel papillomaviruses in free-ranging Iberian bats: no virus–host co-evolution, no strict host specificity, and hints for recombination. *Genome of Biological Evolution.* 6(1):94–104.

Garigliany MM, Hagen RM, Frickmann H, May J, Schwarz NG, Perse A, Jöst H, Börstler J, Shahhosseini N, Desmecht D, Mbunkah HA, Daniel AM, Kingsley MT, Campos Rde M, de Paula VS, Randriamampionona N, Poppert S, Tannich E, Rakotozandrindrainy R, Cadar D, Schmidt-Chanasit J. 2014. Cyclovirus CyCV-VN species distribution is not limited to Vietnam and extends to Africa. *Scientific Reports.* 4:7552.

Ge X, Li J, Peng C, Wu L, Yang X, Wu Y, Zhang Y, Shi Z. 2011. Genetic diversity of novel circular ssDNA viruses in bats in China. *Journal of General Virology.* 92:2646–2653.

Jánoska M, Vidovszky M, Molnár V, Liptovszky M, Harrach B, Benkö M. 2011. Novel adenoviruses and herpesviruses detected in bats. *The Veterinary Journal.* 189:118–121.

Kemenesi G, Dallos B, Görföl T, Estók P, Boldogh S, Kurucz K, Oldal M, Marton S, Bányai K, Jakab F. 2015. Genetic diversity and recombination within bufaviruses: Detection of a novel strain in Hungarian bats. *Infection and Genetic Evolution.* 33:288–292.

Kobayashi S, Sasaki M, Nakao R, Setiyono A, Handharyani E, Orba Y, Rahmadani I, Taha S, Adiani S, Subangkit M, Nakamura I, Kimura T, Sawa H. 2015. Detection of novel polyomaviruses in fruit bats in Indonesia. *Archives of Virology.* 160:1075–1082.

Li L, Shan T, Soji OB, Alam MM, Kunz TH, Zaidi SZ, Delwart E. 2011. Possible cross-species transmission of circoviruses and cycloviruses among farm animals. *Journal of General Virology.* 92:768–772.

Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E. 2010. Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses. *Journal of Virology.* 84:6955–6965.

Li Y, Ge X, Hon CC, Zhang H, Zhou P, Zhang Y, Wu Y, Wang LF, Shi Z. 2010a. Prevalence and genetic diversity of adeno-associated viruses in bats from China. *Journal of General Virology.* 91(Pt 10):2601–2609.

Li Y, Ge X, Zhang H, Zhou P, Zhu Y, Zhang Y, Yuan J, Wang L-F, Shi Z. 2010b. Host range, prevalence, and genetic diversity of adenoviruses in bats. *Journal of Virology.* 84(8):3889–3897.

Lima FE, Cibulski SP, Elesbao F, Carnieli Junior P, Batista HB, Roehe PM, Franco AC. 2013. First detection of adenovirus in the vampire bat (*Desmodus rotundus*) in Brazil. *Virus Genes.* 47(2):378–381.

Lima FEdS, Cibulski SP, Dall Bello AG, Mayer FQ, Witt AA, Roehe PM, d'Azevedo PA. 2015a. A novel chiropteran circovirus genome recovered from a Brazilian insectivorous bat species. *Genome Announcements.* 3(6):01393–15.

Lima FEdS, Cibulski SP, dos Santos HF, Teixeira TF, Varela APM, Roehe PM, Delwart E, Franco AC. 2015b. Genomic characterization of novel circular ssDNA viruses from insectivorous bats in Southern Brazil. *PLoS ONE.* 10(2):e0118070.

Maeda K, Hondo E, Terakawa J, Kiso Y, Nakaichi N, Endoh D, Sakai K, Morikawa S, Mizutani T. 2008. Isolation of novel adenovirus from fruit bat (*Pteropus dasymallus yayeyamae*). *Emerging Infectious Diseases.*14(2):347–349.

Male MF, Kraberger S, Stainton D, Kami V, Varsani A. 2016. Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific flying fox (*Pteropus tonganus*) faeces. *Infection, Genetics and Evolution.* 39:279–292.

Merck Veterinary Manual. 2014. Overview of porcine circovirus diseases. http://www.merckvetmanual.com/mvm/generalized_conditions/porcine_circovirus_diseases/overview_of_porcine_circovirus_diseases.html. Accessed May 16, 2016.

Misra V, Dumonceaux T, Dubois J, Willis C, Nadin-Davis S, Severini A, Wandeler A, Lindsay R, Artsob H. 2009. Detection of polyoma and corona viruses in bats of Canada. *Journal of General Virology.* 90:2015–2022.

Molnár V, Jánoska M, Harrach B, Glávits R, Pálmai N, Rigó D, Sós E, Liptovszky M. 2008. Detection of a novel bat gammaherpesvirus in Hungary. *Acta Veterinaria Hungarica.* 56(4):529–538.

Raut CG, Yadav PD, Towner JS, Amman BR, Erickson BR, Cannon DL, Sivaram A, Basu A, Nichol ST, Mishra AC, Mourya DT. 2012. Isolation of novel adenovirus from *Rousettus leschenaultii* bats from India. *Intervirology.* 55(6):488–490.

Razafindratsimandresy R, Jeanmaire EM, Counor D, Vasconcelos PF, Sall AA, Reynes J-M. 2009. Partial molecular characterization of alpha herpesviruses isolated from tropical bats. *Journal of General Virology.* 90:44–47.

Rector A, Mostmans S, Van Doorslaer K, McKnight CA, Maes RK, Wise AG, Kiupel M, Van Ranst M. 2006. Genetic characterization of the first chiropteran papillomavirus, isolated from a basosquamous carcinoma in an Egyptian fruit bat: the Rousettus aegyptiacus papillomavirus type 1. *Veterinary Microbiology.* 117:267–275.

Sano K, Okazaki S, Taniguchi S, Masangkay JS, Puentespina R Jr, Eres E, Cosico E, Quibod N, Kondo T, Shimoda H, Hatta Y, Mitomo S, Oba M, Katayama Y, Sassa Y, Furuya T, Nagai M, Une Y, Maeda K, Kyuwa S, Yoshikawa Y, Akashi H, Omatsu T, Mizutani T. 2015. Detection of a novel herpesvirus from bats in the Philippines. *Virus Genes.* 51(1):136–139.

Song J-r, Jin Y, Xie Z-p, Gao H-c, Xiao N-g, Chen W-x, Xu Z-q, Yan K-l, Zhao Y, Hou Y-d, Duan Z-j. 2010. Novel human bocavirus in children with acute respiratory tract infection. *Emerging Infectious Diseases.* 16(2):324–327.

Sonntag M, Mühldorfer K, Speck S, Wibbelt G, Kurth A. 2009. New adenovirus in bats, Germany. *Emerging Infectious Diseases.* 15(12):2052–2055.

Tao Y, Shi M, Conrardy C, Kuzmin IV, Recuenco S, Agwanda B, Alvarez DA, Ellison JA, Gilbert AT, Moran D, Niezgoda M, Lindblade KA, Holmes EC, Breiman RF, Rupprecht CE, Tong S. 2013. Discovery of diverse polyomaviruses in bats and the evolutionary history of the Polyomaviridae. *Journal of General Virology.* 94(Pt 4):738–748.

Tse H, Tsang AK, Tsoi HW, Leung AS, Ho CC, Lau SK, Woo PC, Yuen KY. 2012. Identification of a novel bat papillomavirus by metagenomics. *PLoS ONE.* 7(8):e43986.

Vidovszky MZ, Kohl C, Boldogh S, Görföl T, Wibbelt G, Kurth A, Harrach. B. 2015. Random sampling of the Central European bat fauna reveals the existence of numerous hitherto unknown adenoviruses. *Acta Veterinaria Hungarica.* 63(4):508–525.

Watanabe S, Maeda K, Suzuki K, Ueda N, Iha K, Taniguchi S, Shimoda H, Kato H, Yoshikawa Y, Morikawa S, Kurane I, Akashi H, Mizutani T. 2010. Novel betaherpesvirus in bats. *Emerging Infectious Diseases.* 16(6):986–988.

Watanabe S, Ueda N, Iha K, Masangkay JS, Fujii H, Alviola P, Mizutani T, Maeda K, Yamane D, Walid A, Kato K, Kyuwa S, Tohya Y, Yoshikawa Y, Akashi H. 2009. Detection of a new bat gammaherpesvirus in the Philippines. *Virus Genes.* 39(1):90–93.

Wibbelt G, Kurth A, Yasmum N, Bannert M, Nagel S, Nitsche A, Ehlers B. 2007. Discovery of herpesviruses in bats. *Journal of General Virology.* 88(Pt 10):2651–2655.

Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q. 2012. Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. *Journal of Virology.* 86(20):10999–11012.

Zhang H, Todd S, Tachedjian M, Barr JA, Luo M, Yu M, Marsh GA, Crameri G, Wang LF. 2012. A novel bat herpesvirus encodes homologues of major histocompatibility complex classes I and II, C-type lectin, and a unique family of immune-related genes. *Journal of Virology.* 86(15):8014–8030.

Zheng XY, Qiu M, Chen HF, Chen SW, Xiao JP, Jiang LN, Huo ST, Shi TL, Ma LZ, Liu S, Zhou JH, Zhang QH, Li X, Chen Z, Wu Y, Li JM, Guan WJ, Xiong YQ, Ma SJ, Zhong XS, Ge J, Cen SW, Chen Q. 2016. Molecular detection and phylogenetic characterization of bat and human adenoviruses in Southern China. *Vector Borne Zoonotic Diseases.* 16(6):423–427.

Zheng XY, Qiu M, Chen SW, Xiao JP, Ma LZ, Liu S, Zhou JH, Zhang Q-H, Li X, Chen Z, Wu Y, Chen HF, Jiang LN, Xiong YQ, Ma SJ, Zhong XS, Huo ST, Ge J, Cen SW, Chen Q. 2015. High prevalence and diversity of viruses of the subfamily Gammaherpesvirinae, family Herpesviridae, in fecal specimens from bats of different species in southern China. *Archives of Virology.* 161:135–140.

8

REVERSE-TRANSCRIBING BAT VIRUSES AND LARGE-SCALE BAT VIROME STUDIES

8.1 BALTIMORE CLASS VI RETROVIRUSES

8.1.1 Exogenous and endogenous retroviruses and their life-cycles

8.1.1.1 Exogenous retroviruses See Table 8.1 for a list of retroviruses with reported association to bats. Retroviruses (family Retroviridae) are positive-sense, enveloped ssRNA viruses whose genomes are flanked by two characteristic long terminal repeats, remnants of which may be used to detect the presence of ancient, defective endogenous retroviruses (discussed below). One distinctive hallmark of retroviruses is the presence of a reverse transcriptase enzyme, a RNA-dependent DNA polymerase, that reverse-transcribes viral RNA into dsDNA at some point during the viral life cycle. The viral dsDNA is then inserted into a host chromosome by the viral integrase enzyme. Integration of retroviruses may affect host evolution by genomic rearrangements or altered regulation of host gene expression (reviewed by Cui *et al*. 2012a). Viruses integrated into host DNA are termed proviruses and remain latent in the host's chromosomes until reactivated to undergo transcription and translation. The newly synthesized viral RNA and proteins are then assembled at the cell's plasma membrane to form functional, infective virions. The virions are lytic and bud in mass from the surface of the infected cell prior to entering new host cells in a continuation of the viral life cycle. Proviruses may remain embedded in the host chromosomes for many years before

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TABLE 8.1 Baltimore Class VI and Class VII bat viruses and results of bat virome studies

Bat family	Bat common name	Bat species	Virus
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	Endogenous betaretrovirus
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*D. rotundus* endogenous betaretrovirus
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Hepesvirus
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	Sers gammaretrovirus
Rhinolophidae	Aba roundleaf bat	*Hipposideros abae*	Hepesvirus
Rhinolophidae	Noack's roundleaf bat	*Hipposideros caffer ruber*	Hepadnavirus
Vespertilionidae	Savi's pipistrelle	*Hypsugo savii*	Picobirnavirus
Megadermatidae	Greater false vampire bat	*Megaderma lyra*	*Megaderma lyra* retrovirus MlRV
Miniopteridae	Japanese long-fingered bat	*Miniopterus fuliginosus*	Bat hepatitis virus
Miniopteridae	Japanese long-fingered bat	*Miniopterus fuliginosus*	Bocavirus
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	Hepesvirus
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	Hepesvirus
Vespertilionidae	David's myotis	*Mytotis davidii*	Endogenous gammaretrovirus
Vespertilionidae	Little brown bat	*Myotis lucifugus*	Endogenous betaretrovirus
Vespertilionidae	Little brown bat	*Myotis lucifugus*	Endogenous gammaretrovirus
Vespertilionidae	Greater mouse-eared bat	*Myotis myotis*	Bocavirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Ahun nairovirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Picobirnavirus
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Rotavirus
Vespertilionidae	Natterer's bat	*Myotis natteri*	Bornavirus
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Gammaherpesvirus MrGHV-1
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Gammaherpesvirus MrGHV-2
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Gammaretrovirus
Vespertilionidae	Rickett's big-footed myotis	*Myotis ricketti*	Papillomavirus
Mystacinidae	Lesser short-tailed bat	*Mystacina tuberculata*	Calcivirus
Mystacinidae	Lesser short-tailed bat	*Mystacina tuberculata*	Hepesvirus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Bornavirus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Ahun nairovirus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Picobirnavirus

(*Continued*)

TABLE 8.1 (Continued)

Bat family	Bat common name	Bat species	Virus
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Picornavirus
Pteropodidae	Black flying fox	*Pteropus alecto*	Endogenous betaretrovirus
Pteropodidae	Black flying fox	*Pteropus alecto*	Gammaretrovirus
Pteropodidae	Large flying fox	*Pteropus vampyrus*	Endogenous betaretrovirus
Pteropodidae	Large flying fox	*Pteropus vampyrus*	Endogenous gammaretroviruses
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	Gammaretrovirus
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* foamy virus 1
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Rhinolophus affinis* pestivirus 1
Rhinolophidae	Halcyon horseshoe bat	*Rhinolophus alcyone*	Hepadnavirus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Adeno-associated virus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Betaherpesvirus RfBHV-1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Cirovirus RfCV-1
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Endogenous betaretrovirus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	Endogenous gammaretrovirus
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	*R. ferrumequinum* retrovirus
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	Rotavirus
Rhinolophidae	Eastern horseshoe bat	*Rhinolophus megaphyllus*	Endogenous betaretrovirus
Rhinolophidae	Eastern horseshoe bat	*Rhinolophus megaphyllus*	Gammaretrovirus
Rhinolophidae	Pearson's horseshoe bat	*Rhinolophus pearsonii*	Gammaretrovirus
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus*	Gammaretrovirus
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultii*	*Rousettus leschenaultii* retrovirus RlRV
Vespertilionidae	Greater bamboo bat	*Tylonycteris robustula*	Betaherpesvirus TrBHV-1
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	Roundleaf bat hepatitis virus B
Phyllostomidae	Great stripe-faced bat	*Vamipyrodes caraccioli*	Hepesvirus

their reactivation. Those retroviruses which produce infective viruses in this manner are termed exogenous retroviruses and comprise several of the retroviral classes.

Human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2), the causative agents of AIDS, are examples of highly pathogenic exogenous RNA retroviruses of humans and have killed millions of people. Simian immunodeficiency virus is a very similar retrovirus of nonhuman primates. The pathogenic human T-cell leukemia virus and the nonpathogenic foamy viruses of humans and nonhuman primates are other exogenous retroviruses. While foamy viruses are often highly cytopathic in tissue culture, causing rapid syncytium formation and a foam-like vacuolization of cells, they have yet to be proven pathogenic to their host species (Linial 1999).

8.1.1.2 Endogenous retroviruses The majority of retroviruses become an integral part of the host genome as endogenous retroviruses (ERVs). They may be expressed or silent and have complete or partial genomes. The latter are defective viruses that can no longer be reactivated into functional exoviruses. Large numbers of endogenous retroviral elements are situated throughout the chromosomes of most organisms, including bats and humans. ERVs are able to transpose, resulting in multiple copies of that particular ERV being integrated into host chromosomes in either a *cis* or a *trans* fashion. When germline cells are infected, vertical transmission of endogenous retroviruses may occur. The resulting proviral genes function in a Mendelian fashion. Since approximate dates of integration may be determined, ERVs act as "genomic fossils" that allow a glimpse into the evolutionary past. Interestingly, some ERVs have been found integrated into the chromosomes of hosts for whom no exogenous retroviruses have been reported (reviewed by Hayward *et al*. 2013).

Over the course of millions of years, accumulated mutations and production of stop codons degrade the genes of these viruses, rendering almost all ERVs defective and unable to reactivate or function as exogenous retroviruses. Very infrequently, an endogenous retroviral gene will undergo positive selection, presumably due to the ability of the gene product to, in some way, benefit the host species. Additionally, an ERV may, on rare occasions, reactivate. Such occurrences have been linked to cancer or other diseases in humans and animals. The circumstances that stimulate these extremely rare events are not well-defined, giving importance to the study of retroviruses and factors that allow them to become activated and to transfer between species.

8.1.2 Viral polyproteins

Retroviral genomes contain several characteristic polycistronic genes encoding polyproteins that must be cleaved by the viral protease in order to produce functional, individual proteins. These polyproteins include Gag, whose products synthesize internal virion proteins; Pol, whose products are the viral protease, reverse transcriptase, and integrase enzymes; and Env, whose products function as viral envelope proteins. The latter proteins target and bind to the appropriate host cell receptors, fuse with the cell's plasma membrane, and permit entry into the host cells. The protein products of the Pol and Env polyproteins are targets of vaccine candidates. Unfortunately, some exogenous retroviruses, including HIV, mutate at an extremely high rate due to a combination of a sloppy reverse transcriptase and the lack of mechanisms for genomic proof-reading. The high rate of mutation of those retroviruses and their ability to form inactive proviruses permits evasion of the highly specific adaptive immune responses and promotes viral resistance to antiviral agents and potential vaccines.

8.1.3 Retroviral genera

Retroviruses are divided into seven genera: *Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Deltaretrovirus, Epsilonretrovirus, Lentivirus*, and *Spumavirus*. Simple retroviruses encode the structural polyproteins Gag and Env and the functional polyprotein Pol. The genomes of complex retroviruses contain additional genes encoding vital accessory and regulatory proteins that aid in transcription or serve as virulence factors (reviewed by Hayward *et al*. 2013). Interestingly, the coding sequences of some of these retroviral genes overlap and are transcribed using different reading frames.

Betaretroviruses comprise Class II retroviruses, while gammaretroviruses are placed into Class I. The highly pathogenic, immunosuppressive, HIV-1 and HIV-2 are members of the *Lentivirus* genus, while Spumaviruses contain the Class III ERV ("foamy viruses") which have incorporated their DNA into host chromosomes. They are the most abundant class of retroviruses, comprising large amounts of host DNA. In general, their genes are degraded to inactive forms to a much greater extent than that seen in the other classes of retroviruses (reviewed by Zhou *et al*. 2013).

8.1.4 Endogenous gammaretroviruses of bats and other mammals

In a study of North American *Myotis lucifugus*, almost 5% of the genome is composed of ERV-derived sequences, a percentage in line with that of other eutherian mammals, including humans, in whom these sequences comprise about 8% of the genome. The study discovered 362 potentially complete proviruses, almost all of which may be placed in 86 subfamilies (Zhou *et al*. 2013). By far, the majority of the complete proviruses were integrated into the *M. lucifugus* genome during the last 25 million years, with 64% having integrated in the last 10 million years. The more recently integrated proviruses represent members of all three major ERV classes (Zhou *et al*. 2013). A copy of a Class I ERV subfamily and a copy of an apparently functional Class II family member suggest that these viruses might be replication-competent and produce infectious viral particles. Endogenous gammaretroviral sequences were also detected in *Myotis davidii, Myotis brandtii*, and *Eptesicus serotinus*, all of the Vespertilionoidae family (Zhou *et al*. 2013; Zhou & Feschotte 2015).

RNA from endogenous gammaretroviruses was detected in the frugivorous Leschenault's rousette (*Rousettus leschenaultii* retrovirus, RlRV) and the insectivorous greater false vampire bat (*Megaderma lyra* retrovirus, MlRV) (Cui *et al*. 2012a). Since both retroviruses contain a large deletion in the *pol* gene, they must be defective retroviruses. Phylogenetic analysis shows that the *R. leschenaultii* retrovirus is most closely related to porcine endogenous retroviruses (70% nucleotide similarity), while the *M. lyra* retrovirus is most closely related to rodent endogenous virus (72% nucleotide similarity), koala retrovirus, and gibbon ape leukemia virus. The genomes of *M. lucifugus* and *Pteropus vampyrus* contain multiple copies ($n = 57$ and $n = 50$, respectively) of defective endogenous retroviral forms related to the above two bat retroviruses. *M. lucifugus* carries group A, B, and C endogenous gammaretroviruses, while group C retroviruses are present in the *P. vampyrus* genome.

An analysis of high quality Pol sequences from approximately 8000 Class I and Class II ERVs from 69 mammalian genomes found that the retroviruses in bats and rodents combined produce the major phylogenetic diversity of both viral classes (Cui *et al*. 2015).

Bats' genomes carry two-fold and fourteen-fold lower copy numbers of Class I and Class II ERVs, respectively, than that of rodents, but have comparable or greater, phylogenetic diversity. This study supports the contention that rodents are more likely to have served as originators of mammalian retroviruses, while bats are better able to receive retroviruses from other mammalian hosts. The novel bat gammaretrovirus, *Rhinolophus ferrumequinum* retrovirus, derived from the bats' brains may have its origin in tree shrews since Pol and Gag phylogenetic trees place this virus basal to all other extant mammalian gammaretroviruses (Cui *et al*. 2012b, 2015). Gammaretroviral sequences were also detected in the following bat species: *Rhinolophus pusillus, Rhinolophus pearsoni, Rhinolophus megaphyllus, Rhinolophus affinis, Myotis ricketti*, and *Pteropus alecto* (Cui *et al*. 2012b).

In a search for cross-species transmission of ERVs, the full genome of *M. lucifugus* was compared with those of known gammaretroviruses of other mammalian species. While transmission most often occurs between closely related species, this study found that the most significant gammaretroviral hits crossed mammalian orders and were with the domestic cat (>80% nucleotide), the Amur tiger, and the Chinese pangolin ("the scaled anteater") (Zhou & Feschotte 2015). These animals represent the orders Chiroptera, Carnivora, and Pholidota, respectively. Interestingly, no close relatives to the *M. lucifugus* retrovirus were seen in any members of the four other bat families or five other families of carnivores, suggesting that this virus was acquired by the various host species horizontally and independently, perhaps by cat predation of bats and pangolins. Amplification of copy numbers of a given ERV may occur by either retrotransposition or by reinfection. The latter is typically evidenced by the lack of a functional Env protein and is faster than the amplification seen in endogenous retroviruses with this gene still intact. The copy numbers of the *M. lucifugus*-related retroviruses are as follows: for full-length ERVs (containing both long-terminal repeats), 88 for the tiger, 3 for the cat, 48 for *M. davidii*, 51 for *M. brandtii*, 204 for *M. lucifugus*, and 2 for the pangolin. The copy numbers were higher for those containing only a single long-terminal repeat: 744 (tiger), 67 (cat), 1042 (*M. davidii*), 948 (*M. brandtii*), 1638 (*M. lucifugus*), and 27 (pangolin) (Zhou & Feschotte 2015). Interestingly, after the entry for this gammaretrovirus into cats and bats, increases in copy numbers appear to have primarily involved retrotransposition in cats, while both retrotransposition and reinfection appear to have occurred in the vesper bats, in which the retrovirus can be divided into three subfamilies (Zhou & Feschotte 2015).

8.1.5 Betaretroviruses of bats and other mammals

The *Betaretrovirus* genus consists of type B and D groups of exogenous and endogenous retroviruses, which lead to cancer or immunodeficiency in animals, and the human endogenous retrovirus-K (HERV-K) group of ERVs. The latter group is linked to human breast, ovarian, and prostate cancers, in addition to autoimmune diseases (reviewed by Hayward *et al*. 2013). A study of endogenous betaretroviruses in the genomes and transcriptomes of Australian Mega- and Microchiroptera found eight distinct subgroups, one of which acquired its *env* gene from a member of the type C *Gammaretrovirus* genus. Betaretroviral mRNA was found in transcriptomes of *P. alecto, R. megaphyllus*, and *R. ferrumequinum*, including a full-length genomic transcript from the former. Since all genes from this transcript contained mutations that would render the resulting proteins nonfunctional, it probably is a defective retrovirus (Hayward *et al*. 2013).

P. vampyrus and *M. lucifugus* contain a wide range of full-length transcripts. A diverse set of novel open reading frames (ORFs) of unknown function was also discovered. These bat viruses appear to have been present in bat genomes for over 30 million years. Since phylogenetic analysis clusters the bat viruses with extant betaretroviruses of divergent mammalian lineages, their distribution does not appear to be highly restricted by host species barriers, unlike the case in gammaretroviruses (Hayward *et al*. 2013).

The common vampire bat from Mexico carries a type D endogenous betaretrovirus, *Desmodus rotundus* endogenous betaretrovirus (Escalera-Zamudio *et al*. 2015). This bat retrovirus is a low-copy-number provirus. While its *pol* and *env* retroviral core elements contain several stop codons, the *gag* and protease gene ORFs do not and thus may code for functional proteins. The retrovirus contains sequences related to those found in the genome of *Carollia perspicillata*, a frugivorous phyllostomid bat, corresponding to the CpERV-_5_AC138156 betaretrovirus, with which it shares 75% total genomic similarity. It also is homologous to a betaretrovirus in the common brown rat genome (*Rattus norvegicus*) as well as to a betaretrovirus from a New World squirrel monkey, having 72% total nucleotide similarity, even though the latter contains a type C retroviral *env* (Escalera-Zamudio *et al*. 2015). Interestingly, no sequences of the *D. rotundus* endogenous betaretrovirus were found in another vampire bat, *Diphylla ecaudata*, suggesting that viral entry occurred subsequent to divergence of these similar bat species. Phylogenetic analysis indicates that for the Gag and Pol trees, the *D. rotundus* virus clusters with that from the squirrel monkey, forming a sister clade to the β5 group rodent retroviruses. A different phylogenetic pattern was seen using the *env* gene. These viruses form a discrete cluster, with an Australian common brushtail possum betaretrovirus at a basal position, diverging from a gammaretrovirus. The remaining β5 rodent and Megachiroptera ERVs diverge from a gibbon ape leukemia gammaretrovirus, suggesting a recombination event occurred in the *env* gene (Escalera-Zamudio *et al*. 2015). The most recent common ancestor estimation for the squirrel monkey/*D. rotundus*/*C. perspicillata* retroviral lineage indicates that the oldest provirus is that from *D. rotundus*, while the integrity of the squirrel monkey genome suggests that its proviruses were more recently active and transmissible. An exogenous member of this retroviral group may still be present in Latin America and may have been transmitted to other host species (Escalera-Zamudio *et al*. 2015).

8.2 EVIDENCE OF ANCIENT ENDOGENOUS VIRUS GENOMIC ELEMENTS IN BAT CHROMOSOMES

Belyi *et al*. (2010) searched for evidence of the presence of ancient elements of non-retroviral single-stranded RNA viruses embedded into the genomes of a 48 vertebrate species, including *M. lucifugus*. Nearly 80 such integrations were found, with almost half of the tested vertebrate species possessing integrated elements in their genomes. Interestingly, almost all of the integrated elements' sequences were related to two viral families of ssRNA (–) viruses, Bornaviruses and Filoviruses, both from the order Mononegavirales (Belyi *et al*. 2010). Interestingly, Bornaviruses replicate in the nucleus, while Filoviruses do so in the host cell's cytoplasm. By contrast, endogenous sequences of the influenza virus were not found in genomes of these vertebrates, even though it also undergoes nuclear replication.

It should be noted that all of the reported endogenous elements in this study have only 30–50% identity with virus proteins from their proposed present-day counterparts. This could reflect the effects of the high mutation rate found in RNA viruses over a long time period or may indicate that the endogenous sequences originated from other closely related, extinct viruses.

8.2.1 Endogenous bornavirus genomic elements in bat chromosomes

Bornaviruses are neurotropic viruses which produce six proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), RNA-dependent RNA polymerase (L), and accessory protein (X). They are the causative agents of Borna disease, a fatal neurologic disease of horses, sheep, and birds. Fragments of bornaviral origin have been found integrated into genomes of several mammalian species, including primates, suggesting an ancient origin of exogenous bornaviruses (reviewed by Cui & Wang 2015).

Endogenization of bornaviral elements has been reported in genomes of some bats, humans, and birds. The integrated bornavirus elements include EBLLs (endogenous bornavirus-like L elements). Fragments of EBLLs and endogenous bornavirus-like N elements (EBLNs) are embedded in the genomes of the little brown bat (*M. lucifugus*), Natterer's bat (*Myotis nattereri*), David's myotis (*M. davidii*), the big brown bat (*Eptesicus fuscus*), and the common pipistrelle (*Pipistrellus pipistrellus*) (Calisher *et al*. 2006; Belyi *et al*. 2010; Horie *et al*. 2010; Taylor *et al*. 2011; Dacheux *et al*. 2014).

A more recent search of ten bat genomes found further evidence of an evolutionary relationship between endogenous bornaviral elements and bats, particularly vesper bats (Cui & Wang 2015). Several viral element types (EBLL, EBLN, EBLG, and EBLM) were discovered in the following bat genomes: EBLN elements in *Rhinolophus ferrumquinum*, *M. lyra, Eidolon helvum, M. brandtii*, and *Pteronotus parnellii*; EBLL in *P. parnellii* and *M. brandtii*; EBLM in *P. parnellii*; and EBLG in *E. fuscus*. Surprisingly, the genome of *E. fuscus* harbored a nearly complete L protein sequence which lacked stop codons (Cui & Wang 2015). Megachiropterans carried low ($n \leq 2$) or no EBLL copies and 1–2 copies of EBLN, while microchiropterans carried higher EBLL copy numbers (6–17 copies). The latter bat suborder also appears to have had frequent bornaviral invasions of EBLLs and either frequent invasions of EBLN or small-scale segmental duplication viral integration sites (Cui & Wang 2015). LINE-1 (long interspersed nuclear element-1) plays a role in EBLL integration. The fact that megachiropterans have less LINE-1 activity than microchiropterans may at least partially explain the relatively low numbers of EBLLs and EBLNs in this bat suborder. EBLL infiltration is also more robust in bats than in other vertebrate orders.

8.2.2 Endogenous Ebola and Marburg virus genomic elements in bat chromosomes

Endogenous elements related to both the Lake Victoria Marburg virus *NP* gene and the Reston Ebolavirus *VP35* gene are present in the genomes of *Myotis* species microbats and *NP*-like elements in *E. fuscus*, indicating a very long-term relationship between certain filoviruses and some groups of bats (Belyi *et al*. 2010; Taylor *et al*. 2011). Parametric simulations suggest that positive selective pressure for the maintenance of

the ORF of the *VP35*-like genes in these bats is present and has been active for an estimated 13.4 million years. The *VP35*-like gene does not appear to be expressed, however. The ORF for the *NP*-like genes, by contrast, has been disrupted (Taylor *et al*. 2011).

The above studies are particularly relevant since several species of megachiropterans, including *Hypsignathus monstrosus, Epomops franqueti*, and *Myonycteris torquata*, as well as the *Mops condylurus* megachiropteran, have been implicated as potential natural reservoirs for exogenous Ebola viruses that cause life-threating disease in humans and other primates. The Reston Ebolavirus, however, is extremely pathogenic to nonhuman primates, but not to humans. It has been suggested that the presence of endogenous viral elements may promote host resistance to infection with the currently circulating related exovirus (Belyi *et al*. 2010). The low degree of identity between the endogenous elements and the related exoviruses, taken together with the high degree of specificity of the adaptive immune system, call for future research into the underlying mechanisms of the proposed protection.

8.3 HEPADNAVIRUSES – BALTIMORE CLASS VII REVERSE-TRANSCRIBING DNA VIRUSES

The family Hepadnaviridae is composed of enveloped, spherical viruses with small, circular, partially double-stranded DNA with four overlapping ORFs. Its genome is the smallest found in DNA viruses. The negative strand is longer than the positive strand and has a protein covalently attached to its 5′ end. The shorter, positive strand contains an RNA oligonucleotide at its 5′ end. The family contains two genera: *Orthohepadnavirus*, whose members infect hepatocytes and cause hepatitis in mammals; and *Avihepadnavirus*, whose members cause hepatitis in birds. *Orthohepadnavirus* has been divided into two clusters: one composed of primate hepatitis viruses; and the other of rodent hepatitis viruses (reviewed by He *et al*. 2013a). See Table 8.1 for a list of hepadnaviruses with reported association to bats.

8.3.1 Human hepatitis B virus

Human hepatitis B virus (HBV) is a blood-borne orthohepadnavirus that targets the liver, causing histopathologic changes typical of hepatitis, cirrhosis, and hepatocellular carcinoma. Viral infection is prevalent in humans, with 40% of the world's population infected currently or in the past, yet the animal reservoir remains unknown. Even though an effective vaccine is available, nearly 2 billion people are infected with HBV, leading to an annual death toll of 600 000 persons. This virus is believed to have entered humans at least 15 000 years ago.

Since orthohepadnaviruses often cause chronic infections, they provide a persistent source of viruses for secondary infection and enable virus maintenance in their host population. Chronic infection occurs at a high rate following neonatal infection, 80–90% for human HBV. These viruses are also highly infectious horizontally between hosts and vertically (up to 90% of offspring of hepatitis B-positive mothers are infected) (reviewed by Rasche *et al*. 2016). The strong hepatotropism of human HBVs is related to the strict expression of its receptor, sodium taurocholate cotransporting polypeptide, in hepatocytes. This greatly reduces viral integration into germ cells prior to vertical transmission (reviewed by Rasche *et al*. 2016).

HBV strains are divided into nine strictly human-associated genotypes (A–I). Other strains are found in nonhuman primates: chimpanzees, gorillas, gibbons, orangutans, and woolly monkeys in South America. The latter appears to be most closely related to ape or human viruses. Primate HBV strains do not typically infect humans. Very few nonprimate orthohepadnaviruses have been reported. These are distantly related to HBV and include HBV of woodchucks, California ground squirrels, and arctic squirrels in limited regions of North America. They are host-specific and are unable to infect human hepatocytes.

8.3.2 Orthohepadnaviruses and bats

A metagenomic study of orthohepadnaviruses from livers of Japanese long-fingered bats (*Miniopterus fuliginosus*) detected novel bat hepatitis viruses forming an independent cluster within *Orthohepadnavirus* (He *et al*. 2013a). The prevalence of the bat hepatitis viruses was 2.2–4.7% ($n=640$). The full genomes of the bat viruses had 63.1–65.3% and 33.9–34.8% identity to members of *Orthohepadnavirus* and *Avihepadnavirus*, respectively, suggesting that they compose a new species. While no evidence of hepatitis viruses was seen in other tested insectivorous bats, such as *Hipposideros armiger* ($n=8$), *Rhinolophus ferrumequinum* ($n=176$), *Myotis chinensis* ($n=11$), *M. lyra* ($n=6$), and *Hipposideros fulvus* ($n=12$) (He *et al*. 2013a), the number of tested animals in these species is generally quite low and so viruses with low prevalence of infection might have been missed.

A search for bat hepadnaviruses was conducted from 2002 to 2011 utilizing highly sensitive nested PCR to test 3080 sera specimens from 54 bat species from 11 bat families in Panama, Brazil, Gabon, Ghana, Germany, Papua New Guinea, and Australia (Drexler *et al*. 2013). Viral DNA was detected in ten specimens, including three novel hepadnaviruses, which exist in co-ancestral relation to human HBV (Drexler *et al*. 2013). Liver samples ($n=5$) all contained high levels of virus, as did the lungs of one bat tested. The prevalence of infection in those bat species carrying hepadnavirus DNA were as follows: 9.3% of the frugivorous tent-making bat (*Uroderma bilobatum*) from Panama ($n=54$), 7.9% of the insectivorous Noack's roundleaf bat (*Hipposideros* cf. *ruber*) ($n=51$), and 6.3% of the insectivorous Halcyon horseshoe bat (*Rhinolophus alcyone*) ($n=6$) from Gabon. The hepadnaviruses were named TBHBV, RBHBV, and HBHBV, respectively (Drexler *et al*. 2013). Infection of bats with these hepadnaviruses resembles human infection with HBV, including inflammatory leukocyte infiltrations of the liver. Pseudotyped viruses expressing surface proteins of one of the bat hepadnaviruses are able to infect human liver cells *in vitro* using the hepatitis B-specific human receptor. Up to 18.4% of tested bat sera contained antibodies against bat hepadnaviruses. All bat viruses varied in their nucleotide sequences by at least 35% from sequences of any previously reported hepadnavirus (Drexel 2013). Only TBHBV is able to infect human hepatocytes *in vitro*. While unlikely, if zoonotic transmission were to occur, TNHBV is the most likely of bat hepadnaviruses to do so.

Given the large extent of genetic diversity of extant bat hepadnaviruses in comparison with other hosts, these viruses may have had a long period of evolution in bats. New World rodents also carry a very diverse group of hepadnaviruses. Rasche *et al*. (2016) suggest that New World bats harbored orthohepadnaviruses that were ancestral to human HBV and other primate hepadnaviruses. The primate hepadnaviruses were suggested to

have arisen by multiple host switches of bat and primate viruses. It should be noted, however, that a high degree of host specificity is generally believed to characterize these viruses (Rasche *et al*. 2016). This calls into question whether a hepadnavirus of bats is able to infect primates.

8.4 LARGE-SCALE BAT VIROME STUDIES

8.4.1 Bat virome studies in North America

A metagenomics study of 390 000 sequence reads from bat guano in the southwestern US found that the largest proportion of eukaryotic viruses present were those infecting insects and the second largest proportion were viruses infecting plants and fungi, probably reflecting the diet of insectivorous bats or that of ingested insects. The third largest group (less than 10%) was composed of viruses related to other viruses which infect mammals or birds. Numerous novel mammalian virus sequences were detected as well, including adenoviruses, adenovirus-associated viruses, astroviruses, coronaviruses, a highly divergent kobuvirus, and parvoviruses. While many unclassified viruses were also detected in the guano, none of these were closely related to known human pathogens (Li *et al*. 2010).

A report of the bat virome in the northeastern US tested fecal, oral, urine, and tissue samples from bats residing in a tunnel that is cohabitated by seven to ten bat species. The viromes of 41 individuals of three common North American bat species, big brown bats (*E. fuscus*), tricolored bats (*Perimyotis subflavus*), and little brown myotis (*M. lucifugus*), were determined. Eukaryotic viral sequences were discovered that were similar to a novel bat alphacoronaviruses (*E. fuscus*) and multiple betaherpesviruses, in addition to many sequences from insect and plant viruses (Donaldson *et al*. 2010). As in the above study, no known emerging human viruses were present, although some sequences were distantly related to human viruses, such as rotavirus, enterovirus, and coronaviruses.

8.4.2 Bat virome studies in Europe

A French study of viromes from pooled liver, lung, and brain of five species of insectivorous bats found that sequences from vertebrate viruses predominated, especially viral sequences from mammals. Most of these bats displayed unusual behavior prior to death. The principal viral families identified were Bunyaviridae, Flaviviridae, Herpesviridae, Poxviridae, Reoviridae, and Retroviridae (Dacheux *et al*. 2014). Of the assigned viral contigs, 10% represented virus of invertebrates (primarily insects), 8% were from plants or fungi, 3% from protozoans, and 8% were bacteriophages. Some of the viruses were novel, including Ahun nairovirus, a member of the primarily tick-transmitted genus Nairovirus of the family Bunyaviridae, from lung tissue of *Myotis mystacinus* and *P. pipistrellus*. A novel rotavirus, distantly related to Rotavirus A, an enteric virus, was detected in lung samples from *M. mystacinus*. A gammaretrovirus, named the Sers gammaretrovirus, was discovered in lung tissue from the serotine bat (*Eptesicus serotinus*). Sequences from a novel bornavirus were detected in brain tissue from *P. pipistrellus* and *Myotis nattereri*, a novel adenovirus from *M. natteri*, and a novel picobirnavirus from *P. pipestrellus*, *M. mystacinus*, and *Hypsugo savii* (Dacheux *et al*. 2014).

8.4.3 Bat virome studies in Asia and Southeast Asia

In a study of viromes from pharyngeal and anal swab samples from 216 bats in China, representing 11 insectivorous species, eukaryotic viruses included those infecting insects, plants, fungi, and mammals (Wu *et al*. 2012). Partial or complete genome sequences of novel mammalian viruses composed 9% of the sequence reads and comprised the following: herpesviruses, papillomaviruses, a bocavirus, a circovirus, a picornavirus, a pestivirus, a foamy virus, astroviruses, adenoviruses, and adeno-associated viruses. The viral sequences had only low genetic similarity with previously reported viruses. The novel herpesviruses discovered were two betaherpesviruses from *Rhinolophus ferrumequinum* (RfBHV-1) and *Tylonycteris robustula* (TrBHV-1), and two gammaherpesviruses from *Myotis ricketti* (MrGHV-1 and MrGHV-2) (Wu *et al*. 2012). Papillomaviruses infect vertebrate skin and mucosa, causing benign and malignant epithelial tumors in humans and in at least some fruit bats. Papillomavirus genomes were present in *Myotis ricketti* (MrPV-1) and *Miniopterus schreibersii* (MschPV-1). This study also detected circovirus 1 (RfCV-1) in *Rhinolophus ferrumequinum*; a novel Boca virus, a parvovirus, in *Myotis myotis*; and picornavirus 1 in *Ia io*, *Miniopterus schreibersii*, and *Rhinolophus affinis* bats. The picornavirus in *M. schreibersii* is closely related to the *Cardiovirus* genus. Members of this genus are associated with severe diseases in humans, including respiratory and gastrointestinal symptoms and nonpolio acute flaccid paralysis. *Pestivirus* is a genus in the family *Flaviviridae*. All previous PestVs were found in animals in the order *Artiodactyla* and cause severe infections in hoofed mammals of that order. A pestivirus was additionally found in *Rhinolophus affinis* (RaPestV-1) in this study. The nonpathogenic foamy viruses (spumaviruses), Class VII DNA retroviruses discussed above, are known to infect cattle, cats, horses, and primates, including humans. A foamy virus was also found in *Rhinolophus affinis* bats (RaFV-1). *Astroviridae* infect many mammals, including humans, and cause gastroenteritis. One novel astrovirus was found in *Myotis ricketti*, one in *Rhinolophus sinicus*, thirteen in *Miniopterus schreibersii*, and one in *Tylonycteris robustula* (Wu *et al*. 2012). Two bat coronaviruses were also detected in *R. affinis* and *M. schreibersii*. These viruses had been previously reported in *Miniopterus magnater* and *M. pusillus* in Hong Kong.

A separate study of the fecal microbiome of 281 insectivorous and frugivorous bats from 20 common Chinese bat species found that the most frequently identified bat viruses were endogenous retroviruses, especially among members of the insectivorous *Hipposideridae* bat family (Yuan *et al*. 2014). Over half of the 100 retrovirus contigs, however, contained stop codons, indicating that they were defective viruses. Many phages were also detected, primarily those parasitizing enteric bacteria.

A study of the virome of thoracic and abdominal organs (laryngopharynx, trachea, lung, heart, liver, spleen, stomach, gut, kidney and bladder) of bats in Myanmar found that 45% of the viral contigs were related to vertebrate viruses, 28% to insect viruses, 27% to phages and less than 0.5% to plant viruses (He *et al*. 2013b). These results differ from those of other metagenomic studies of bat viromes, in which bacteriophages, plant, and insect viruses predominated. It should be noted that the other studies did not obtain their bat samples from the above organs. This is particularly important in viruses similar to hepadnaviruses, which are found strictly in blood and are not normally secreted by the fecal or oral routes. Fourteen families of novel vertebrate bat viruses were discovered in the Myanmar bats: Adenoviridae (mastadenoviruses), Alloherpesviridae (ictaluriviruses), Herpesviridae (Cytomegaloviruses,

mardiviruses, and roseoloviruses), Papillomaviridae (alphapapillomaviruses), Polyomaviridae (polyomaviruses), Poxviridae (orthopoxviruses), Picobirnaviridae (picobirnaviruses), Hepadnaviridae (orthohepadnaviruses), Retroviridae (deltaretroviruses), Circoviridae (circoviruses), Parvoviridae (dependoviruses and bocaviruses), Astroviridae (mamastroviruses), Flaviviridae (hepaciviruses), and Picornaviridae (kobuviruses and enteroviruses). Interestingly, over 10 000 contigs were related to Hepadnaviridae, which shared 70% nucleotide identity with human HBV. *Miniopterus fuliginosus* was found to harbor astroviruses and bocaviruses and *Rhinolophus ferrumequinum* carried astroviruses, adenoviruses, and adeno-associated viruses (He *et al*. 2013b). It will be interesting to compare the diversity of bat viromes to that found in other groups of mammals, especially rodents and primates, in order to determine whether or not bats have a wider diversity of viruses or whether this viral diversity is common among mammals. Several studies have already examined the viromes of rodents or healthy humans (Phan *et al*. 2011; Rascovan *et al*. 2016).

8.4.4 Bat virome studies in Oceania

A 2015 study examined the metavirome in guano from four roosts of the insectivorous lesser short-tailed bat (*Mystacina tuberculata*) in New Zealand, one of the island's two extant, indigenous bats. They found that most of the DNA and RNA viral reads were from bacteriophages and many of the viruses of eukaryotes were from Flaviviridae, a group known to infect insects (Wang *et al*. 2015). The vertebrate viruses discovered in this study included two novel papillomavirus sequences grouping with deltapapillomaviruses; a novel bat polyomavirus most closely related to a polyomavirus of South American bats; a calcivius whose closest relative is a norovirus; and a novel hepevirus distantly related to the cut-throat trout virus (Wang *et al*. 2015). No conserved genetic elements were detected for either adenoviruses or poxviruses, although it is possible that the bats may harbor a virus similar to molluscum contagiosum virus, a human pathogen. *M. tuberculata*, the long-tailed bat (*Chalinolobus tuberculatus*), and one extinct bat species are the only indigenous terrestrial mammals in New Zealand. They and their viromes were in isolation for millions of years, so large differences with viral sequences from other locations are to be expected. It is not known whether the New Zealand bat viruses have the potential to spill over into humans or domestic animals or to cause disease in these potential new hosts.

8.5 CONCLUSIONS

ERVs are members of Baltimore Class VI RNA retroviruses. ERVs have integrated into host chromosomes over the course of millions of years and exist in an inactive, proviral form. Over long periods of time, the vast majority of the ERVs have undergone mutations that have rendered them defective and unable to form exogenous retroviruses that replicate and infect other cells. ERVs do, however, on rare occasions transpose and form multiple copies in the original or other chromosomes of the host cell. ERVs are found in most animal species, including humans and bats.

Studies of ERVs in *M. lucifugus* discovered 362 potentially complete proviruses, including members of all three ERV classes. These compose about 5% of its genome, similar to the 8% found in humans. Other species of bats also harbor integrated gammaretroviruses, most of which are defective, but others appear to be replication-competent

and may be able, under appropriate circumstances, to form infectious, exogenous retroviruses. A large study of the *pol* gene from 69 mammalian genomes revealed that ERVs of bats and rodents combined have the major phylogenetic diversity among tested mammal species. While bats have lower copy numbers of Class I and Class II ERVs than rodents, they have comparable or greater, phylogenetic diversity.

Bornaviruses cause fatal neurological diseases in horses, sheep, and birds. Several genetic elements of apparent bornavirus origin are found in the genomes of bats, humans, and birds. Copy numbers of these elements differ between major groups of bats. Several bat genomes also carry endogenous filoviral elements that are related to genes from Lake Victoria Marburg virus and the Reston Ebolavirus. The former is highly pathogenic, while the latter is not pathogenic to humans. The ORF corresponding to at least one Marburg virus gene has been disrupted, so there is little chance that it may be reactivated.

Baltimore Class VII hepadnaviruses include the blood-borne human HBV, which causes hepatitis, cirrhosis of the liver, and hepatocellular carcinoma. There are nine strictly human-associated hepatitis B genotypes, as well as other strains in primates that do not typically infect humans. A large study that tested 54 bat species from locations throughout the world found several bat hepatitis viruses. Their nucleotide sequences vary by 35% or more from those of other hepadnaviruses, including human HBV. Only one of the tested bat hepatitis viruses was able to infect human hepatocytes *in vitro*. Additionally, liver samples from five bats all contained high levels of virus and experimentally infected bats developed inflammatory leukocyte infiltrations of the liver similar to those present in humans. It has been proposed that primate hepadnaviruses arose as a result of multiple host switches between bat and primate viruses. However, the high degree of viral host specificity together with large genetic differences with primate hepadnaviruses calls this proposition into question.

Several large studies of bat viromes have been performed using material from thoracic and abdominal organs, pharyngeal and anal swab samples, or fecal samples. These studies had very differing results: some finding that the majority of viruses infect insects, plants, or fungi, while other findings suggested that the majority of viruses were ERVs, phages, or viruses that infect vertebrates. All of the studies noted a large degree of viral diversity and found numerous novel viruses. In order to interpret these findings, it will be necessary to conduct a number of such viromic studies of other groups of animals, particularly those which have close contact with humans, to determine whether the large amount of diversity in bat viromes is unique or is common among other groups of animals.

REFERENCES

Belyi VA, Levine AJ, Skalka AM. 2010. Unexpected inheritance: Multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes. *PLoS Pathology.* 6:e1001030.

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. 2006. Bats: Important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews.* 19:531–545.

Cui J, Tachedjian G, Tachedjian M, Holmes EC, Zhang S, Wang L-F. 2012a. Identification of diverse groups of endogenous gammaretroviruses in mega- and microbats. *Journal of General Virology.* 93:2037–2045.

Cui J, Tachedjian G, Wang L-F. 2015. Bats and rodents shape mammalian retroviral phylogeny. *Scientific Reports.* 5:16561.

Cui J, Tachedjian M, Wang L, Tachedjian G, Wang L-F, Zhang S. 2012b. Discovery of retroviral homologs in bats: implications for the origin of mammalian gammaretroviruses. *Journal of Virology.* 86(8):4288–4293.

Cui J, Wang L-F. 2015. Genomic mining reveals deep evolutionary relationships between bornaviruses and bats. *Viruses.* 7:5792–5800.

Dacheux L, Cervantes-Gonzalez M, Guigon G, Thiberge J-M, Vandenbogaert M, Maufrais C, Caro V, Bourhy H. 2014. A preliminary study of viral metagenomics of French bat species in contact with humans: identification of new mammalian viruses. *PLoS ONE.* 9(1):e87194.

Donaldson EF, Haskew AN, Gates JE, Huynh J, Moore CJ, Frieman MB. 2010. Metagenomic analysis of the viromes of three North American bat species: viral diversity among different bat species that share a common habitat. *Journal of Virology*. 84(24):13004–13018.

Drexler JF, Geipel A, König A, Corman VM, van Riel D, Leijten LM, Bremer CM, Rasche A, Cottontail VM, Maganga GD, Schlegel M, Müller MA, Adam A, Klose SM, Carneiro AJ, Stöcker A, Franke CR, Gloza-Rausch F, Geyer J, Annan A, Adu-Sarkodie Y, Oppong S, Binger T, Vallo P, Tschapka M, Ulrich RG, Gerlich WH, Leroy E, Kuiken T, Glebe D, Drosten C. 2013. Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. *Proceedings of the National Academy of Science USA*. 110(40):16151–16156.

Escalera-Zamudio M, Mendoza MKZ, Heeger F, Loza-Rubio E, Rojas-Anaya E, Méndez-Ojeda ML, Taboada B, Mazzoni CJ, Arias CF, Greenwood AD. 2015. A novel endogenous betaretrovirus in the common vampire bat (*Desmodus rotundus*) suggests multiple independent infection and cross-species transmission events. *Journal of Virology.* 89:5180–5184.

Hayward JA, Tachedjian M, Cui J, Field H, Holmes E, Wang L-F, Tachedjian G. 2013. Identification of diverse full-length endogenous betaretroviruses in megabats and microbats. *Retrovirology*.10:35.

He B, Fan Q, Yang F, Hu T, Qiu W, Feng Y, Li Z, Li Y, Zhang F, Guo H, Zou X, Tu C. 2013a. Hepatitis virus in long-fingered bats, Myanmar. *Emerging Infectious Diseases.* 19(4):638–640.

He B, Li Z, Yang, Zheng J, Feng Y, Guo H, Li Y, Wang Y, Su N, Zhang F, Fan Q, Tu C. 2013b. Virome profiling of bats from Myanmar by metagenomic analysis of tissue samples reveals more novel mammalian viruses. *PLoS ONE.* 8(4):e61950.

Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T, Ikuta K, Jern P, Gojobor T, Coffin JM, Coffin JM, Tomonaga K. 2010. Endogenous non-retroviral RNA virus elements in mammalian genomes. *Nature.* 463:84–87.

Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E. 2010. Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses. *Journal of Virology.* 84(14):6955–6965.

Linial ML. 1999. Foamy viruses are unconventional retroviruses. *Journal of Virology.* 73(3): 1747–1755.

Phan TG, Kapusinszky B, Wang C, Rose RK, Lipton HL, Delwart EL. 2011. The fecal viral flora of wild rodents. *PLoS Pathology.* 7(9):e1002218.

Rasche A, de Carvalho Dominguez Souza, Drexler JF. 2016. Bat hepadnaviruses and the origins of primate hepatitis B viruses. *Current Opinion in Virology.* 16:86–94.

Rascovan N, Duraisamy R, Desnues C. 2016. Metagenomics and the human virome in asymptomatic individuals. *Annual Review of Microbiology*.70:125–141.

Taylor DJ, Dittmar K, Ballinger MJ, Bruenn JA. 2011. Evolutionary maintenance of filovirus-like genes in bat genomes. *BMC Evolutionary Biology.* 11:e336.

Wang J, Moore NE, Murray ZL, McInnes K, White DJ, Tompkins DM, Hall RJ. 2015. Discovery of novel virus sequences in an isolated and threatened bat species, the New Zealand lesser short-tailed bat (*Mystacina tuberculata*). *Journal of General Virology*. 96:2442–2452.

Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q. 2012.Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. *Journal of Virology.* 86(20):10999–11012.

Yuan L, Li M, Li L Monagin C, Chmura AA, Schneider BS, Epstein JH, Mei X. 2014. Evidence for retrovirus and paramyxovirus infection of multiple bat species in China. *Viruses.* 6:2138–2154.

Zhuo X, Feschotte C. 2015. Cross-species transmission and differential fate of an endogenous retrovirus in three mammal lineages. *PLoS Pathogens.* 11(11):e1005279.

Zhuo X, Rho M, Feschotte C. 2013. Genome-wide characterization of endogenous retroviruses in the bat *Myotis lucifugus* reveals recent and diverse infections. *Journal of Virology.* 87(15):8493–8501.

III

BACTERIAL INFECTIONS OF BATS

9

ARTHROPOD-BORNE BACTERIAL INFECTIONS OF BATS

9.1 INTRODUCTION

Ticks, fleas, and other arthropods together with the bacteria they carry are responsible for much human misery and many deaths. This chapter includes several bacterial genera (*Bartonella, Borrelia*, and *Rickettsia*) that infect humans, their domestic animals, and bats, sometimes with severe consequences, while other times accompanied by only minor symptoms in immunocompetent humans and animals. See Table 9.1 for a list of arthropod-borne bacteria associated with bats. Many of the resulting diseases are considered to be emerging infections or infections that were previously undiagnosed and are just coming to our awareness due to their devastating effects upon the growing population of immunocompromised people. Since bats are infected with several known human pathogens from these genera or by closely related bacteria, the potential for zoonotic transmission exists. Bats, even immunocompetent animals, are not themselves always protected from severe illness or death, suggesting that they are not ideal reservoir hosts for these bacteria. The possibility remains, however, that bats may play some role in human infection and disease, in addition to the detrimental effects upon the bats themselves.

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 9.1 Arthropod-borne bacteria associated with bats

Family	Bat common name	Bat species	Bacteria
Phyllostomidae	Geoffroy's tailless bat	*Anoura geoffroyi*	*Bartonella* sp.
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	*Bartonella* sp.
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Bartonella* sp.
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	*Bartonella* sp.
Phyllostomidae	Toltec fruit-eating bat	*Artibeus toltecus*	*Bartonella* sp.
Phyllostomidae	Lesser Antillean fruit-eating bat	*Brachyphylla cavernarum*	*Bartonella* sp.
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	*Bartonella* sp.
Phyllostomidae	Chestnut short-tailed bat	*Carollia castanea*	*Bartonella* sp.
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Bartonella* sp.
Phyllostomidae	Sowell's short-tailed bat	*Carollia sowelli*	*Bartonella* sp.
Molossidae	Nigerian free-tailed bat	*Chaerephon nigeriae*	*Bartonella* sp.
Emballonuroidea	African sheath-tailed bat	*Coleura afra*	*Bartonella* sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Bartonella* sp.
Pteropodidae	Madagascan fruit bat	*Eidolon dupreanum*	*Bartonella* sp.
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella clarridgeiae*
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella elizabethae*
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella henselae*
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella quintana*
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella* strain E1–105
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Bartonella vinsonii vinsonii*
Pteropodidae	Epauletted fruit bats	*Epomorphorus* sp.	*Bartonella* sp.
Pteropodidae	Wahleberg's epauletted fruit bat	*Epomophorus wahlbergi*	*Bartonella* sp.
Pteropodidae	Wahleberg's epauletted fruit bat	*Epomophorus wahlbergi*	Spotted fever group *Rickettsia*
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Spotted fever group *Rickettsia*
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	Relapsing fever group *Borrelia*
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Neorickettsia risticii*
Vespertilionidae	Butterfly bat	*Glauconycteris variegata*	Spotted fever group *Rickettsia*
Pteropodidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Bartonella* sp.
Hipposideridae	Greater roundleaf bat	*Hipposideros armiger*	*Bartonella* sp.
Hipposideridae	Giant roundleaf bat	*Hipposideros commersoni*	*Bartonella* sp.
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatui*	*Bartonella* sp.
Megadermatidae	Lesser false vampire bat	*Megaderma spasma*	*Bartonella* sp.
Megadermatidae	Greater false vampire bat	*Megaderma lyra*	*Bartonella* sp.
Pteropodidae	Ratanaworabhan's fruit bat	*Megaerops niphanae*	*Bartonella* sp.

(*Continued*)

TABLE 9.1 (Continued)

Family	Bat common name	Bat species	Bacteria
Phyllostomidae	Little big-eared bat	*Micropterus microtis*	*Bartonella* sp.
Miniopteridae	Natal long-fingered bat	*Miniopterus natalensis*	*Bartonella* sp.
Miniopteridae	Natal long-fingered bat	*Miniopterus natalensis*	*Rickettsia* sp.
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersii*	*Bartonella* sp.
Phyllostomidae	Leach's single-leafed bat	*Monophyllus redmani*	*Bartonella* sp.
Vespertilionidae	Daubenton's bat	*Myotis daubentonii*	*Bartonella mayotimonensis*
Vespertilionidae	Hairy-legged myotis	*Myotis keaysi*	*Bartonella* sp.
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Neorickettsia risticii*
Vespertilionidae	Whiskered bats	*Myotis mystacinus*	*Bartonella mayotimonensis*
Vespertilionidae	Northern myotis	*Myotis septentrionalis*	*Bartonella mayotimonensis*
Emballonuroidea	Egyptian slit-faced bat	*Nycteris thebaica*	*Bartonella* sp.
Emballonuroidea	Egyptian slit-faced bat	*Nycteris thebaica*	Spotted fever group *Rickettsia*
Vespertilionidae	Common noctule	*Nystulus noctula*	*Bartonella* sp.
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	*Bartonella* sp.
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	*Bartonella* sp.
Vespertilionidae	Pipistrelle bats	*Pipistrellus* sp.	Relapsing fever group *Borrelia*
Vespertilionidae	Pipistrelle bats	*Pipistrellus* sp.	*Bartonella* sp.
Phyllostomidae	Greater broad-nosed bat	*Platyrrhinus vittatus*	*Bartonella* sp.
Mormoopidae	Lesser naked-back bat	*Pteronotus davyi*	*Bartonella* sp.
Pteropodidae	Madagascan flying fox	*Pteropus rufus*	*Bartonella* sp.
Rhinolophidae	None	*Rhinolophus chaseli*	*Bartonella* sp.
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	*Bartonella* sp.
Rhinolophidae	Egyptian fruit bat	*Rousettus aegyptiacus*	*Bartonella* sp.
Vespertilionidae	African yellow bat	*Scotophilus dinganii*	Spotted fever group *Rickettsia*
Phyllostomidae	Little yellow-shouldered bat	*Sturnira lilium*	*Bartonella* sp.
Phyllostomidae	Talamancan yellow-shouldered bat	*Sturnira mordax*	*Bartonella* sp.
Rhinolophidae	Persian trident bat	*Triaenops persicus*	*Bartonella* sp.
Phyllostomidae	Northern little yellow-eared bat	*Vampyressa thyone*	*Bartonella* sp.
Phyllostomidae	Bidentate yellow-eared bat	*Vampyriscus bidens*	*Bartonella* sp.

9.2 *BARTONELLA*

Most *Bartonella* species are hemotropic, facultative intracellular parasites of mammalian erythrocytes and endothelial cells. These Gram-negative bacteria are highly adapted to their specific mammalian hosts (Lei & Olival 2014). More than 20 *Bartonella* species have been identified and greater than half of these are pathogenic to humans. Disease manifestations in infected humans include cat scratch disease fever (*Bartonella henselae*), trench fever (*Bartonella quintana*), Carrión disease (*Bartonella bacilliformis*), potentially fatal endocarditis (*B. henselae, B. quintana, Bartonella elizabethae*, and *Bartonella mayotimonensis*), and bacillary angiomatosis and bacillalry peliosis hepatitis (*B. henselae, B. quintana*) (reviewed by Beltz 2011). Disease severity ranges from a self-limiting, short-term fever to fatal systemic disease affecting the cardiovascular and nervous systems with hepatosplenic involvement. Immunosuppressed people are especially at risk for severe disease. Known *Bartonella* reservoir hosts include rodents, cats, and dogs. Many of these bacteria are transmitted to mammals by arthropod vectors, including sandflies, lice, fleas, ticks, and mites. Bats host a variety of ectoparasites, such as bat flies, fleas, hard and soft ticks, and mites. One or more of these arthropods may transmit *Bartonella* to bat hosts (reviewed by Kosoy *et al*. 2010). Bats are home to extremely diverse species of *Bartonella* and multiple species may circulate in the same bat community or even in an individual bat (Bai *et al*. 2015).

9.2.1 *Bartonella* in bats from Asia

Anh *et al*. (2015) studied the incidence of *Bartonella* species in southern Vietnam using conventional PCR. The infection rate among insectivorous bats was 33% for *Hipposideros armiger* ($n=6$), 60% for *Hipposideros larvatui* ($n=50$), 50% for *Megaderma spasma* ($n=2$), 40% for *Rhinolophus chaseli* ($n=5$), and 29% of *Rhinolophus sinicus* ($n=7$). The infection rate among frugivorous bats was 50% for *Megaerops niphanae* ($n=2$), while no *Bartonella* species were detected in 14 *Cynopterus sphinx* bats. The one *Megaderma lyra* carnivorous bat that was studied was positive for *Bartonella* DNA. The overall prevalence of *Bartonella* infection detected in southern Vietnam bats was 35.5%, comparable with that in Kenya (30.2%) and Guatemala (33.0%). *Bartonella* species are also common in rats from southern Vietnam. Of note, despite the presence of *Bartonella* species in rats and bats, no *Bartonella* species have been reported in humans of the region. This is despite close contact between humans and bats through guano farming and consumption of bat meat.

Sequencing and phylogenetic analyses of sequences derived from four bat species from Taiwan revealed a potentially novel *Bartonella* in 42.9% ($n=14$) of *Miniopterus schreibersii*. These bacteria differ from other known *Bartonella* species (82.2–91.2% similarity) (Lin *et al*. 2012). In contrast, no *Bartonella* were found in the 35 *Pipistrellus abramus* bats studied.

9.2.2 *Bartonella* in bats from Africa

Real time PCR was used to screen members of five Nigerian bat species ($n=148$) and 24 of their associated bat flies (*Cyclopodia greeffi*) for the presence of *Bartonella* species DNA (Kamani *et al*. 2014). *Bartonella* DNA was present in 51.4% of the bat

blood samples and in 41.7% of *C. greeffi*. Detection of *Bartonella* DNA varied among bat species: 55.7% of the *Eidolon helvum* ($n=79$), 53.3% of *Epomorphorus* species ($n=30$), 54.5% of *Micropterus* species ($n=11$), 66.7% of *Rhinolophus* species ($n=12$), and 12.5% of *Chaerephon nigeriae* ($n=16$). When only studying *C. greeffi* from *Bartonella*-positive bats, *Bartonella* DNA was found in 71.4% of *C. greeffi* from *E. helvum* and 100% of *C. greeffi* from *Micropterus* and *Rhinolophus* species. The bacteria were also isolated from 15.5% of the bat blood samples. The incidence of culture-positive blood varied greatly among bat species: 45.5% of *Micropterus* species, 25% of *Rhinolophus* species, 15.2% of *E. helvum*, 10% of *Epomophorus* species, and none of the tested *C. nigeriae*. It should be noted that all of the tested bat species are frugivorous except *C. nigeriae*, which are insectivorous (Kamani *et al*. 2014).

Bartonella from these Nigerian bats formed three distinct clusters, in agreement with *Bartonella* from bats and bat flies in Kenya and Ghana, respectively. *Bartonella* species are also present in bats from Peru, Guatemala, Kenya, and the UK (Concannon *et al*. 2005; Kosoy *et al*. 2010; Bai *et al*. 2011, 2012) and in bat ectoparasites from Egypt, the US, and Ghana (Loftis *et al*. 2005; Reeves *et al*. 2006, 2007; Billeter *et al*. 2012). It is not known whether the strains of *Bartonella* infecting bats cause human illnesses.

When blood of Kenyan bats was examined, *Bartonella* was isolated from 30.2% of 331 bats representing 13 species from 9 genera (Kosoy *et al*. 2010). The prevalence of infection among these bat species is as follows: 26.1% of straw-colored fruit bat (*E. helvum*; $n=88$), 21.0% of Egyptian fruit bats *(Rousettus aegyptiacus*; $n=105$), 44.4% of African sheath-tailed bats *(Coleura afra*; $n=9$), 87.5% of Persian trident bats (*Triaenops persicus*; $n=8$), 25.0% of giant leaf-nosed bats *(Hipposideros commersoni*; $n=4$), and 56.3% of long-fingered bats (*Miniopterus* species; $n=87$). No *Bartonella* were detected in the 23 *Epomophorus* bats tested. It is not known if any *Bartonella* from the bats are able to infect or cause illness in humans.

A study of blood samples from 384 insectivorous or frugivorous bats from 29 species of eight bat families in South Africa and Swaziland found that 3.3% of the bats harbored *Bartonella* DNA. The *Bartonella*-positive bat species were *Miniopterus natalensis, Nycteris thebaica* (one individual bat co-infected with *Rickettsia*), *Epomophorus wahlbergi*, and *R. aegyptiacus* (Dietrich *et al*. 2016). The Nycteribiidae flies from *R. aegyptiacus* also harbored *Bartonella* species. The bacteria from *E. wahlbergi* shared 100% sequence similarities with the human pathogen *B. elizabethae* (308 base pairs analyzed). *Bartonella* prevalence is high in both healthy and HIV-positive human subjects (9.5 and 22.5%, respectively) in this area of southern Africa. The high host-specificity of the insect vectors may indicate that the global risk of spillover of bat-borne *Bartonella* to humans may be low. In Africa, however, the greater level of human activity in caves may lead to a greater risk of bat-to-human spillover and, due to the high prevalence of HIV infection, the consequences of such zoonotic spillover may be grim, especially in those people who are malnourished or infected by other parasites.

Bartonella tamiae was detected in 60% ($n=10$) of tested bat spleens from northeastern Algeria and in 72.7% ($n=11$) of their associated Nycteribiidae flies. *B. tamiae* was also found in 63.2% of bat-specific *Ixod vespertilionis* ticks ($n=19$). Human infections with this *Bartonella* species led to headaches, myalgia, anemia, as well as mild liver function abnormalities. Additionally, 15.8% of the *I. vespertilionis* had DNA from *Coxiella burnetii*, which causes Q fever in humans (Leulmi *et al*. 2016).

A study of 79 *Bartonella* isolates from *E. helvum* from seven African countries revealed that these bats were infected with six distinct *Bartonella* phylogenetic lineages (*E1–E5* and *Ew*) that correspond to unique bacterial species. Lineage *Ew* composed more than 25% of the isolates and had little genetic variability, suggesting fairly recent origin (Bai *et al*. 2015). *E. helvum* roosts may contain millions of bats, often near human populations. These bats are frequently hunted as bush meat, increasing human contact with bat blood. Interestingly, a study in Ghana found that all serum samples from 335 people having close contact with *E. helvum* bats were culture-negative for *Bartonella henselaei, Bartonella quintana, Bartonella clarridgeiae, Bartonella vinsonii vinsonii, Bartonella elizabethae*, and *Bartonella* strain E1-105, all of which infect *E. helvum* bats (Mannerings *et al*. 2016). The study also could not culture these *Bartonella* species from sera of domestic animals residing beneath bat colonies (5 cats, 23 chickens, 7 cows, 6 dogs, 21 goats, and 8 sheep). One human serum sample was, nevertheless, PCR-positive for *B. clarridgeiae* and one cat was PCR-positive for *B. henselae*. The relative lack of evidence of human infection with bat-associated *Bartonella* species suggests that humans are, at most, rarely infected by *Bartonella* species from *E. helvum* bats (Mannerings *et al*. 2016).

In Madagascar, a novel species of *Bartonella* was found in the blood of 44.8% of the cave-roosting Madagascan fruit bat (*Eidolon dupreanum*; $n=47$). Almost all (99%) of the associated bat flies (*Cyclopodia dubia*) also carried these bacteria ($n=19$) (Brook *et al*. 2015). No *Bartonella* were detected in *Thaumapsylla* fleas present on the same bats ($n=6$), even though fleas are vectors for *B. henselaei*. Given the small sample size, bat fleas cannot be ruled out as vectors for *Bartonella* in bats. Neither *Bartonella* nor ectoparasites were found in sympatrically sampled tree-roosting Madagascan flying foxes (*Pteropus rufus*). Bat flies in *Bartonella* may, therefore, play a major role in transmission of *Bartonella* to at least some species of bats, but may not promote interspecies bacterial transmission.

9.2.3 *Bartonella* in bats from Europe

In Finland, *Candidatus* status species *Bartonella mayotimonensis* have been detected in blood of Daubenton's myotis (*Myotis daubentonii*), the northern myotis (*Myotis septentrionalis*), and whiskered bats (*Myotis mystacinus*). This *Bartonella* species is able to cause endocarditis in humans. A novel *Bartonella* species, *Bartonella naantaliensis* sp. nov., was also present. *Bartonella* was also found in ectoparasites of *M. daubentonii, M. septentrionalis*, and Brandt's bats (*Myotis brandtii*) (Veikkolainen *et al*. 2014). *Bartonella* was detected by molecular means in 8.3% of English bats ($n=60$) from four different species, *M. mystacinus, M. daubentonii*, the common noctule (*Nystulus noctule*), and two members of the *Pipistrellus* genus (Concannon *et al*. 2005).

9.2.4 *Bartonella* in bats from the Americas

Olival *et al*. (2015) cultured *Bartonella* from the blood of 18% of *Artibeus jamaicensis, Brachyphylla cavernarum*, and *Monophyllus redmani* ($n=51$) in a shared roosting in Puerto Rica. The prevalence of *Bartonella* from five sites in Guatemala averaged 33% ($n=118$) and included 21 genetic variants of the bacteria from 13 phylogroups (Bai *et al*. 2011). *Bartonella* prevalence was 88.8% in *Phyllostomus discolor* ($n=9$), 70% in

Pteronotus davyi ($n=10$), 48.4% in *Desmodus rotundus* ($n=31$), 13.3% in *Glossophaga soricina* ($n=15$), 28.6% in *Carollia perspicillata* ($n=14$), and 8.3% in *Sturnira lilium* ($n=12$). Additionally, one of three *Micronycteris microtis* and the only tested *Artibeus toltecus* were infected. Some of the bat species (*C. perspicillata, D. rotundus, P. discolor*, and *P. davyi*) were infected with two to four different *Bartonella* strains.

A recent study of Costa Rican bats detected *Bartonella* species DNA in 33.3% of bats examined. The following bats were infected: *M. microtus, Myotis keaysi, Carollia sowelli, C. perspicillata, Carollia castanea, Artibeus lituratus, A. jamaicensis, Platyrrhinus vittatus, Vampyressa thyone, Anoura geoffroyi, P. discolor, S. lilium*, and *Sturnira mordax* (Judson *et al*. 2015). Additionally, *Bartonella* DNA was detected in 15 of the 23 species of bat flies and, overall, *Bartonella* DNA was seen in 52.7% of the tested bat flies. (See Judson *et al*. 2015 for the complete list.) When bat–bat fly pairs were tested, for 27.2% of the pairs, both bat and its flies contained *Bartonella* DNA ($n=44$), although the *Bartonella* variant found in the bat usually differed from the variant found in its associated fly (Judson *et al*. 2015).

Bartonella was found in 57.9% of Peruvian bats ($n=19$) (Bai *et al*. 2012). The prevalence of bacteria in the positive bat species are as follows: 10% of *A. obscurus* ($n=10$), 12.5% of *Artibeus planirostris* ($n=16$), 100% of *Carollia brevicauda* ($n=2$), 13.8% of *C. perspicillata* ($n=29$), 55.6% of *D. rotundus* ($n=18$), 50% of *G. soricina* ($n=2$), 16.7% of undefined *Myotis* species ($n=6$), 100% of *P. discolor* ($n=2$), 50% of *Phyllostomus hastatus* ($n=2$), 100% of *S. lilium* ($n=1$), and 66.7% of *Vampyriscus bidens* ($n=3$). None of the 10 *Molossus molossus* were positive.

Only limited reports are available for the presence of *Bartonella* in bats in the US. From the Southern US, a survey of 56 *Eptesicus fuscus* identified antibodies to one spotted fever group *Rickettsia* and three relapsing fever group *Borrelia* (Reeves *et al*. 2006). Neither bacterial group was able to be cultured from the blood of the seropositive animals, indicating that the bats were exposed to the bacteria, but not necessarily infected.

9.3 *BORRELIA*

In addition to the Lyme disease agent, *Borrelia burgdorferi, Borrelia* species spirochetes cause tick-borne relapsing fever in humans in western North America and are transmitted by argasid soft ticks. In humans, tick-borne relapsing fever is also linked to infection with *Borrelia hermsii, Borrelia turicatae*, and *Borrelia parkeri* (reviewed in Gill *et al*. 2008). The argasid bat tick, *Carios kelleyi*, feeds upon humans as well as bats and is widely distributed in the US (Gill *et al*. 2004). A novel species of *Borrelia* was found in *C. kelleyi* from central US (Gill *et al*. 2008). The spirochete was most closely related to, but distinct from, *B. turicatae* and *B. parkeri*. Only limited reports are available for the presence of *Borrelia* in bats in the US. As mentioned above (Section 9.2.4), three relapsing fever group *Borrelia* were found in 56 *E. fuscus* in the Southern US (Reeves *et al*. 2006). Neither bacterial group was able to be cultured from the blood of the seropositive animals, indicating that the bats were exposed to the bacteria, but not necessarily infected.

A juvenile female *Pipistrellus* bat from the UK died from fatal borreliosis, caused by spirochetes (Evans *et al*. 2009). The liver had multifocal necrosis and vacuolation of hepatocytes. The lungs were congested and inflamed. Spirochetes were found in the

liver, lungs, spleen, and blood. Sequence analysis of the bat spirochete indicated that it was related to a cluster of *Borrelia* containing *Borrlia recurrentis, Borrelia duttonii*, and *Borrleia crocidurae*, all linked to relapsing fevers in Africa and Asia. A larval short-legged bat tick (*Argas vespertilionis*) was found attached to the infected bat. The tick was near-replete with blood that was heavily infected by spirochetes. These ticks parasitize bats across Europe, southern Asia, and North Africa and also bite humans.

9.4 *RICKETTSIA*

Members of *Rickettsia* and the closely related genus *Orientia* are small, Gram-negative bacilli. These obligate intracellular parasites of eukaryotic cells parasitize arthropods (ticks, lice, fleas, and mites) and mammals, including humans and bats. Transmission of the bacteria to mammals may be via the arthropods' bite or feces entering broken skin. The two bacterial genera differ in in the presence of lipopolysaccharide, peptidoglycan, and a slime layer in *Rickettsia* which are not found in *Orientia* (Walker 1996).

9.4.1 *Rickettsia* and human disease

Rickettsia and similar bacteria cause a number of human diseases, many of which are emerging and are particularly severe in immunocompromised people. Rickettsial diseases in humans result from infection with several members of the bacterial genera *Rickettsia, Orientia, Ehrlichia, Neorickettsia, Neoehrlichia*, and *Anaplasma* (McQuiston 2016). *Rickettsia* species are separated into the spotted fever and typhus groups. *Rickettsia* pathogenic to humans include the following bacteria from the spotted fever group: *Rickettsia aeschlimannii* (causative agent of rickettsiosis), *Rickettsia africae* (African tick-bite fever), *Rickettsia akari (rickettsialpox), Rickettsia amblyommii* ("spotless" Rocky Mountain spotted fever), *Rickettsia conorii* (Mediterranean spotted fever), *Rickettsia felis* (cat flea rickettsiosis), *Rickettsia heilongjiangensis* (Far Eastern spotted fever), *Rickettsia helvetica* (aneruptive fever), *Rickettsia honei* (Flinders Island spotted fever, Thai tick typhus), *Rickettsia japonica* (Japanese spotted fever), *Rickettsia massiliae* (Mediterranean spotted fever-like disease), *Rickettsia monacensis* (Mediterranean spotted fever-like illness), *Rickettsia parkeri* (maculatum infection), *Rickettsia raoultii* (tick-borne lymphadenopathy), *Rickettsia rickettsii* (Rocky Mountain/Brazilian spotted fever), *Rickettsia sibirica* (North Asia or Siberian tick typhus), *R. sibirica mongolotimonae* (lymphangitis-associated rickettsiosis), and *Rickettsia slovaca* (tick-borne lymphadenopathy). The human pathogens from the typhus group are *Rickettsia prowazekii* (epidemic or sylvatic typhus) and *Rickettsia typhi* (murine typhus). The larval mite-borne *Orientia* species, including the human pathogen *Orientia tsutsugamushi*, comprise the scrub typhus group. Tick-borne *Ehrlichia chaffeensis, Ehrlichia muris*, and *Ehrlichia ewingii* cause human ehrlichiosis. *Neorickettsia sennetsu* causes sennetsu fever and is unusual in that its vector is a fish trematode rather than an arthropod. Tick-borne *Neoehrlichia mikurensis* causes human neoehrlichiosis. Tick-borne *Anaplasma phagocytophilum* causes human anaplasmosis. A wide range of arthropods serve as vectors (ticks, mites, fleas, and lice) and a wide range of vertebrates serve as reservoir hosts (rodents, deer, ruminants, domestic dogs and cats, opossums, flying squirrels, fish, and reptiles) (McQuiston 2016).

The above human rickettsial diseases range in severity from mild to life-threatening, but commonly include fever, headache, malaise, nausea, and vomiting, in addition to a maculopapular, vesicular, or petechial rash or eschar at the site of the arthropod bite. Other possible symptoms are: abdominal pain (Rocky Mountain/Brazilian spotted fever); myalgia, lymphadenopathy, and encephalitis (scrub typhus); severe, but nonspecific febrile illness (murine and epidemic typhus); and leukopenia and immunosuppression (ehrlichiosis and anaplasmosis) (McQuiston 2016).

9.4.2 *Rickesttsia* and bats

Rickettsial bacteria have been reported in bats from diverse regions of the world. Some of these bacterial species are known human pathogens, while many others are not. From the Americas, a Brazilian study searched for the presence of rickettsial antigens in 451 bats. Antigenic prevalence was 8.6% for *R. rickettsii*, 9.5% for *R. parkeri*, 7.8% for *R. amblyommii*, and 1.1% for *Rickettsia rhipicephali* (D'Auria *et al*. 2010). *Neorickettsia ristici* are also transmitted to bats in the northern US via infected trematodes. The intestines of 88.9% of little brown bats (*Myotis lucifugus*; $n = 9$) and 80% of big brown bats (*E. fuscus*; $n = 15$) harbored gravid (egg-bearing) *Acanthatrium oregonense* trematodes as well. *N. risticii*, therefore, appears to be vertically transmitted from adult to egg in these trematodes. *N. risticii* DNA was present in blood, liver, or spleen of 43.4% of *E. fuscus* and *M. lucifugus* bats ($n = 53$) (Gibson *et al*. 2005). *Rickettsia* species DNA was also detected in 1.6% of blood samples from bats from South Africa and Swaziland ($n = 384$). The *Rickettsia*-positive bat species were *M. natalensis, N. thebaica, E. wahlbergi, Scotophilus dinganii*, and *Glauconycteris variegate* (Dietrich *et al*. 2016).

9.5 BAT ECTOPARASITES AS BACTERIAL VECTORS

Bats are parasitized by several groups of arthropods belonging to the Acari (ticks and mites), Dermaptera (earwigs), Diptera (true flies), Hemiptera (true bugs), and Siphonaptera (fleas) orders. The ticks are from the families Argasidae (*Ornithodoros* and *Carios* species of soft ticks) and Ixodidae (*Ixodes* and *Amblyomma* species of hard ticks), in addition to bat flies (Bertola *et al*. 2005; Franck *et al*. 2013).

9.5.1 Bacteria from bat flies

Bat flies are bloodsucking ectoparasites residing on bats' fur and wings. Bat flies are divided into two families: wingless, spider-like Nycteribiidae in the Eastern Hemisphere, which are obligate blood-sucking flies; and the more traditional fly-like Streblidae in the Western Hemisphere, with full or reduced wings. Nycteribid flies and mites typically are host-specific, permanent bat ectoparasites (reviewed by Hornok *et al*. 2012). A study of southern Australian nycteribid bat flies (*Nycteribia parilis vicaria* Maa, *Penicillidia oceanica* Bigot, and *Penicillidia tectisentis* Maa) from *M. schreibersii* Kuhl bats found an average of 1.6 ± 0.3 flies per bat with little variation during the course of the year (Archer & Cardinal 2001). These flies did not appear to negatively affect the bats, including the absence of allergic responses.

In Ghana, 39 genotypes of *Bartonella* DNA were found in up to 66.4% of *Cyclopodia greefi greefi* nycteribiid flies removed from *E. helvum* fruit bats. Many of these sequences (65.9%; $n=82$) were similar or identical to *Bartonella* species found in the *E. helvum* bat host (Billeter *et al*. 2012). A separate large, multiyear study encompassing 141 countries and 19 species of bat flies from 20 bat species discovered 26 novel *Bartonella* genotypes in bat fly adults and pupae (Morse *et al*. 2012). It is quite possible that future studies will continue to find more species of bat flies capable of hosting and transmitting *Bartonella* species. Nycteribiid flies also serve as vectors and definitive hosts for members of the protozoan hemosporidian genus *Polychromophilus* (reviewed by Schaer *et al*. 2015).

In the Malagasy region, seven bat fly species from five fly genera (*Eucampsipoda, Penicillidia, Nycteribia, Cyclopodia*, and *Basilia*) were found to parasitize bats. The bacteriome of these bat flies was then examined (Wilkinson *et al*. 2016). In general, the following bacterial groups were associated with all bat flies: *Alphaproteobacteria*, 17% of the total number of DNA sequences; *Betaproteobacteria*, 3% of the sequences; and *Gammaproteobacteria*, 78% of the sequences. Of the *Alphaproteobacteria* sequences detected, 55% were from *Wolbachia*, 26% from *Bartonella*, and 17% from *Rickettsia*. The *Betaproteobacteria* had the closest similarity to members of the family *Neisseriaceae*, which include *Neisseria gonorrhoeae* and *Neisseria meningitides*, the causative agents of gonorrhea and meningococcal meningitis in humans, respectively. Of the *Gammaproteobacteria*, 99% of the sequences were from the order *Enterobacteriales*, primarily endosymbiotic, *Arsenophonus*-like organisms (Wilkinson *et al*. 2016).

In the above Malagasy region study, *Bartonella* species were strongly associated with all five of the above mentioned bat fly genera. *Eucampsipoda theodori* derived from *Rousettus obliviosus* from the Union of the Comoros had significantly higher proportions of *Bartonella* sequences than those of its sister fly, *Eucampsipoda madagascarensis*, derived from *Rousettus madagascariensis* from Madagascar (Wilkinson *et al*. 2016). *Bartonella* species were found on the following bat flies/bats: *C. dubia* flies from *Eidon dupreanum* bats; *Basilla* species flies from *Scotophilus marovaza* and *Scotophilus robustus* bats; *Eucampsida theodori* flies from *R. obliviosus* bats; *Penicillidia* cf. *fulbida* flies and *Miniopterus griveaudi* bats; *Penicillidia leptothrinax* flies and *Miniopterus manavi, M. griveaudi*, and *Miniopterus aelleni* bats; and *Nycteribia stylidiopsis* flies from *Miniopterus gleni* and *M. griveaudi* bats (Wilkinson *et al*. 2016).

9.5.2 Bacteria from bat ticks

Unlike bat flies, bat ticks and fleas tend to be less host-specific, transiting between host species, and, therefore, more likely to cause interspecies transmission of microbes. Of note, some bat-adapted soft ticks also bite humans (reviewed by Hornok *et al*. 2012). Several species of hard ticks, however, feed only on bats: *Ixodes simplex* found primarily on *M. schreibersii, Ixodes kopsteini* on *Tadarida* species mastiff bats, and *Ixodes vespertilionis*, which feeds on several bat species. *A. vespertilionis* commonly parasitizes Old World bats and is known to aggressively attack humans and domestic animals. It also carries pathogens of human and animal importance, such as *Borrelia burgdoferi sensu lato* (Lyme disease) and *C. burnetii* (Q fever) (reviewed by Burazerović *et al*. 2015).

Five species of hard ticks (*I. vespertilionis, I. simplex, Ixodes ariadnae, I. ricinus*, and *Ixodes trianguliceps*) and one soft tick (*A. vespertilionis*) have been recorded on bats in Poland and Slovakia. The first three of these ticks are specific to bats, while *I. ricinus* and

I. trianguliceps parasitize a wide range of vertebrates, including bats on rare occasions (reviewed in Piksa *et al*. 2016). In a survey in Poland, *I. vespertilionis* were found parasitizing the following bat species: *Rhinolophus hipposideros, Myotis myotis, Myotis nattereri, Myotis emarginatus, M. brandtii*, and *M. mystacinus*, while *I. ricinus* were found attached to *R. hipposideros, M. myotis, Myotis bechsteinii*, and *M. daubentonii* (Piksa *et al*. 2016). A previous study found that 26.8% of *R. hipposideros* bats in two nursery colonies in attics ($n = 810$) carried *I. vespertilionis*. The larval stage of tick was found most frequently, followed by the nymph, and adult females (Piksa *et al*. 2014). Prevalence and infestation intensity was highest in the spring and lowest in July and August. *I. vespertilionis* has additionally been found on *Myotis blythii, Myotis alcathoe, Plecotus auritus, Nyctalus noctula*, and *Pipistrellus pygmeus* in Europe (reviewed by Burazerović *et al*. 2015).

A survey of 491 *I. vespertilionis* derived from bats or cave walls in southern Poland failed to detect DNA of *B. burgdorferi sensu lato* complex, *Rickettsia* species, or *A. phagocytophilum*. DNA from *R. helvetica*, a spotted fever group rickettsia, was found in three of eight *Ixodes ricinus* attached to *R. hipposideros* or *M. myotis* bats and *Borrelia garinii* of the *B. burgdorferi s. l.* complex in one tick from *M. daubentonii*. *A. phagocytophilum* were not found on this small sampling of *I. ricinus* (Piksa *et al*. 2016).

In France, four of five *A. vespertilionis* ticks collected from the floor of a bat-infested attic were infected with *Borrelia* species CPB1, an agent of relapsing fever. Three of five ticks carried the *Rickettsia* species AvBat, a new member of spotted fever group rickettsiae, as well as the novel *Ehrlichia* species AvBat (Socolovschi *et al*. 2012). *A. vespertilionis* have been known to occasionally bite humans and, therefore, may carry the above bacteria from bats to humans

In Hungry, *Bartonella* were detected in a female *I. vespertilionis* hard tick on the wall of a cave used by *Rhinolophus* species and/or *M. myotis* bats (Hornok *et al*. 2012). The Hungarian study additionally detected *Bartonella* DNA in the eight-combed bat flea (*Ischnopsyllus octactenus*), two species of mites (*Steatonyssus occidentalis* and *Spinturnix myoti*), and nycetribid flies (Hornok *et al*. 2012). None of the bat ectoparasites in this study were infected with *B. burgdorferi, Francisella tularensis, C. burnetii*, haemoplasmas, or *A. phagocytophilum*. Reeves *et al*. (2007) detected *Bartonella* species in the bat flea *Sternopsylla texanus*, which are associated with *Myotis lucifuigus* and *Tadarida brasiliensis* bats.

In the Central Balkans, three species of ticks were found on bats (Burazerović *et al*. 2015). The majority of the ticks recovered were: *I. simplex* (158 ticks) found primarily on *M. schreibersii* (156), but two ticks were on *Rhinolophus euryale*; *I. vespertilionis* (6) on *R. euryale, Rhinolophus ferrumequinum, M. schreibersii*, and *M. mystacinus*; and one larval *A. vespertilionis* on *Pipistrellus pipistrellus*.

A study encompassing 52 species of bats in Malaysia found 0.4% harbored ticks of the *Amblyomma, Dermacentor, Ixodes*, and *Ornithodoros* genera (Ahamad *et al*. 2013). Over 10% of these bats also bore 15 species of mesostigmatid mites: *Ancystropus eonycteris, Ancystropus zeleborii, Echinonysus nasutus, Laelaps aingworthae, Laelaps nuttalli, Laelaps sanguisugus, Laelaps sculpturatus, Longolaelaps longulus, Longolaelaps whartonii, Meristaspis lateralis, Meristaspis macroglossi, Paraperiglischrus rhinolophinus, Spinturnix acuminatus, Spinturnix americanus*, and *Spinturnix bakeri*. Six species of chiggers were present on 14.7% of the studied bats: *Gahrliepia fletcheri, Riedlinia lipoxena, Trombigastia cadei, Walchiella impar, Walchiella oudemansi*, and *Whartonia caobangensis*.

In a study of the *C. kelleyi* bat ticks in central US, *Rickettsia* DNA was found in 90.3% of soft ticks ($n = 31$) (Loftis *et al*. 2005). Based upon sequences of several genes,

this bacterium may be a novel *Rickettsia* species of the spotted fever group. Of note, these ticks occasionally feed upon human in addition to their typical bat hosts, opening the possibility of passing this rickettsial species to humans. This report nevertheless, did not find any *Coxiella* or *A. phagocytophilum* DNA in the tested bats. A separate study in the same area found a relapsing fever spirochete in the coxal fluid and salivary glands of a *C. kelleyi* tick. The spirochete is closely related to, but distinct from, *B. turicatae*. *Borrelia johnsonii* was proposed as the name for this novel spirochete (Schwan *et al*. 2009). In the southwestern US, seven species of bats were found to be parasitized by larvae of the argasid ticks *Ornithodoros kelleyi* and *Ornithodoros rossi*. The pallid bat (*Antrozous pallidus*) and the big brown bat (*E. fuscus*) harbored both tick species (Steinlein *et al*. 2001).

A study in French Guiana examined DNA from the larvae of *Ornithodoros hasei*, a species of Argasidae soft ticks, from healthy insectivorous/carnivorous lesser bulldog bats (*Noctilio albiventris*) ($n = 32$) for the presence of *Rickettsia, Bartonella*, and *Borrelia* species, as well as *C. burnetii* (Tahir *et al*. 2016). Soft tick larvae were found on 37.5% of the bats and the number of larvae per bat ranged from 4 to 67 (average infestation $= 29.5 \pm 21$). DNA from the above bacteria was not detected in any of the tested samples with the exception of an undefined *Rickettsia* species, detected in 28.9% of the ticks ($n = 107$) from twelve tick-infested bats. This rickettsia differs from the other members of the spotted fever group and is tentatively named *Candidatus* Rickettsia wissemanii. It is phylogenetically closely related to the nonpathogenic *Rickettsia peacockii* and to the human pathogen *R. rickettsia*. *Rickettsia bellii*, the most common rickettsia of ticks in the US, is found in both *Argas* and *Ornithodoros* genera. The spotted fever group member *Rickettsia hoogstraalii* is also regularly found in *Ornithodoros* and *Haemaphysalis* ticks (reviewed by Tahir *et al*. 2016).

9.6 CONCLUSIONS

Bats, humans, and other mammals are impacted by non-mosquito, arthropod-borne bacterial infections, including diseases caused by members of the *Bartonella, Borrelia*, and *Rickettsia* genera. While many of the resulting diseases are mild or transient, immunocompromised humans and bats are more likely to develop severe or life-threatening illnesses. Some of the diseases, however, are highly pathogenic even for immunocompetent individuals or bats, making bats unlikely reservoirs for the responsible bacteria. While bats might, nevertheless, play a small role in human infections, other mammals may be more likely to serve as reservoir hosts.

Several groups of ectoparasites are present on bats. These include bat flies, mites, soft and hard ticks, fleas, true flies, true bugs, and earwigs. A wingless group of bat flies and mites are highly host-specific, permanent residents of their host, while other groups of bat flies are more mobile. Studies of bat flies from seven genera revealed that they harbor a very diverse range of bacteria, including upwards of 60 *Borrelia* species alone. Some bat ticks and fleas are less host-specific and are thus more likely to cause cross-species bacterial transfer. While some hard ticks feed only on bats, some species of bat soft ticks may also feed on humans or domestic animals, including *A. vespertilionis*, which carries *B. b. sensu lato* (the causative agent of Lyme disease) and *C. burnetii* (Q fever). Some of the ticks and mites taken from bats harbored some pathogenic bacteria species, but given the host-specific nature of some of these arthropods, the presence of

these bacteria in bat ectoparasites does not necessarily indicate that indirect transmission of these bacteria to humans is likely to occur or, if transmission does occur, it may do so only on rare occasions.

Members of the *Bartonella* species are obligate, intracellular bacteria, which tend to be highly host-specific. Rodents, cats, and dogs are, nevertheless, known to serve as reservoirs for *Bartonella* species. These bacteria are typically transmitted to humans via ectoparasites, as is the case in bats. *Bartonella* species are found in insectivorous and frugivorous bats, as well as in the small numbers of carnivorous bats tested. The overall prevalence of *Bartonella* in bats from various locations around the world is 30–36%, but may reach 50–60%, even in regions in which human disease has not been reported. The bat fly vectors have an even higher level of infection than that seen in bats, 70–100%, depending upon the species of fly tested and their location. Analysis of a short sequence of DNA from a *Bartonella* species infecting *E. wahlbergi* revealed 100% similarity to that of the human pathogen *B. elizabethae*, one of the causative agents of life-threatening endocarditis. However, the insect vectors are highly host-specific, making indirect zoonotic infection from bats unlikely to be a significant cause for concern. *B. tamiae*, which is responsible for typically mild disease in humans, was also been found in the spleens of 60% of African bats, but the sample size was quite low ($n = 10$). *E. helvum* also hosts a variety of *Bartonella* species that are human pathogens, but people and domestic animals of the region were seronegative for these bacteria.

Several species of *Borrelia* cause tick-borne relapsing fever in humans. Some of these ticks also infect bats. Despite the paucity of data concerning *Borrelia* in bats, these bacteria are capable of causing severe disease in their bat hosts, as evidenced by the fatal infection of a *Pipistrellus* bat with severe liver and lung disease. An infected bat tick was found attached to the bat.

Obligate intracellular bacilli of the *Rickettsia, Orientia*, and similar genera are transmitted between host species by arthropods or, in the case of *N. risticii*, by trematodes. They are the causative agents of a wide variety of human diseases whose manifestations range from mild in most people to severe in immunocompromised populations. The diseases are divided into the spotted fever and typhus groups. Antibody prevalence to the human pathogens *R. rickettsia, R. parkeri*, and *R. amblyommii* range from 8 to 10% in some species of bats, indicating exposure to bacterial antigens, but not necessarily infection. Some bats are also seropositive for *R. rhipicephali* and *N. risticii*, not known to be pathogenic to humans. DNA from the latter bacilli has also been found in blood, livers, or spleens of more than 40% of tested *E. fuscus* and *M. lucifugus*. Many vertebrates are known to serve as reservoir hosts for pathogenic and nonpathogenic members of these genera, including rodents, deer, ruminants, domestic dogs and cats, opossums, flying squirrels, fish, and reptiles. Bats, however, have not been implicated in indirect zoonotic disease transmission.

REFERENCES

Ahamad M, Ibrahim H, Bujang MK, Sah SA, Mohamad N, Nor SM, Ahmad AH, Ho TM. 2013. A survey of acarine ectoparasites of bats (Chiroptera) in Malaysia. *Journal of Medical Entomology.* 50(1):140–146.

Anh PH, Van Cuong N, Son NT, Tue NT, Kosoy M, Woolhouse MEJ, Baker S, Bryant JE, Thwaites G, Carrique-Mas JJ, Rabaa MA. 2015. Diversity of *Bartonella* spp. in bats, Southern Vietnam. *Emerging Infectious Diseases*. 21(7):1266–1267.

Archer MS, Cardinal BR. 2001. Seasonal reproduction and host infestation rates for nycteribiids of the large bentwing bat. *Medical and Veterinary Entomology.* 5:452–454.

Bai Y, Hayman DTS, McKee CD, Kosoy MY. 2015. Classification of *Bartonella* strains associated with straw-colored fruit bats (*Eidolon helvum*) across Africa using a multi-locus sequence typing platform. *PLoS Neglected Tropical Diseases.* 9(1):e0003478.

Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, Turmelle A, Ellison J, Garcia DL, Estevez A, Lindblade K, Rupprecht C. 2011. *Bartonella* spp. in bats, Guatemala. *Emerging Infectious Diseases*. 17(7):1269–1272.

Bai Y, Recuenco S, Gilbert AT, Osikowicz LM, Gómez J, Rupprecht C, Kosoy MY. 2012. Prevalence and diversity of *Bartonella* spp. in bats in Peru. *American Journal of Tropical Medicine and Hygiene*. 87(3):518–523.

Beltz LA. 2011. *Bartonella* infections. In: *Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey-Bass: San Fransico, CA, pp. 97–116.

Bertola PB, Aires CC, Favorito SE, Gracioll G, Amaku M, Pinto-da-Rocha R. 2005. Bat flies (Diptera: Streblidae, Nycteribiidae) parasitic on bats (Mammalia: Chiroptera) at Parque Estadual da Cantareira, São Paulo, Brazil: Parasitism rates and host-parasite associations. *Memórias do Instituto Oswaldo Cruz*. 100:25–32.

Billeter SA, Hayman DTS, Peel AJ, Baker K, Wood JLN, Cunningham A, Suu-Ire R, Dittmar K, Kosoy MY. 2012. *Bartonella* species in bat flies (Diptera: Nycteribiidae) from western Africa. *Parasitology.* 139:324–329.

Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, Kosoy MY, Dittmar L. 2015. *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. *PLoS Neglected Tropical Diseaseas*. 10(2):e0003532.

Burazerović J, Ćakić S, Mihaljica D, Sukara R, Ćirović D, Tomanovic S. 2015. Ticks (Acari: Argasidae, Ixodidae) parasitizing bats in the central Balkans. *Experimental and Applied Acarology.* 66:281–291.

Concannon R, Wynn-Owen K, Simpson V, Birtles RJ. 2005. Molecular characterization of haemoparasites infecting bats (Microchiroptera) in Cornwall, UK. *Parasitology*. 131:489–496.

D'Auria SR, Camargo MC, Pacheco RC, Savani ES, Dias MA, da Rosa AR, de Almeida MF, Labruna MB. 2010. Serologic survey for rickettsiosis in bats from São Paulo city, Brazil. *Vector Borne Zoonotic Diseases.* 10(5):459–463.

Dietrich M, Tjale MA, Weyer J, Kearney T, Seamark ECJ, Nel LH, Monadjem A, Markotter W. 2016. Diversity of *Bartonella* and *Rickettsia* spp. in bats and their blood-feeding ectoparasites from South Africa and Swaziland. *PLoS ONE*. 11(3):e0152077.

Evans NJ, Bown K, Timofte D, Simpson VR, Birtles RJ. 2009. Fatal borreliosis in bat caused by relapsing fever spirochete, United Kingdom. *Emerging Infectious Diseases.* 15(8):1331–1333.

Franck F, Münster J, Schulze J, Liston A, Klimpel S. 2013. Macroparasites of Microchiroptera: bat ectoparasites of Central and South America. In: *Bats (Chiroptera) as Vectors of Diseases and Parasites. Facts and Myths*. S Klimpel and H Melhorn (eds). Springer-Verlag: Berlin, pp. 87–130.

Gibson KE, Rikihisa Y, Zhang C, Martin C. 2005. *Neorickettsia risticii* is vertically transmitted in the trematode *Acanthatrium oregonense* and horizontally transmitted to bats. *Environmental Microbiology.* 7(2):203–212.

Gill JS, Rowley WA, Bush PJ, Viner JP, Gilchrist MJ. 2004. Detection of human blood in the bat tick *Carios* (*Ornithodoros*) *kelleyi* (Acari: Argasidae) in Iowa. *Journal of Medical Entomology.* 41:1179–1181.

Gill JS, Ullmann AJ, Loftis AD, Schwan TG, Raffel SJ, Schrumpf ME, Piesman J. 2008. Novel relapsing fever spirochete in bat tick. *Emerging Infectious Diseases.* 14(3):522–523.

Hornok S, Kovács R, Meli ML, Gönczi E, Kontschán J, Gyuranecz M, Dán Á, Molnár V. 2012. First detection of bartonellae in a broad range of bat ectoparasites. *Veterinary Microbiology.* 159:541–543.

Judson SD, Frank HK, Hadly EA. 2015. Bartonellae are prevalent and diverse in Costa Rican bats and bat flies. *Zoonoses and Public Health.* 62:609–617.

Kamani J, Baneth G, Mitchell M, Mumcuoglu KY, Gutiérrez R, Harrus S. 2014. *Bartonella* species in bats (Chiroptera) and bat flies (Nycteribiidae) from Nigeria, West Africa. *Vector-Borne and Zoonotic Diseases.* 14(9):625–632.

Kosoy M, Bai Y, Lynch T, Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Breiman RF, Rupprecht CE. 2010. *Bartonella* spp. in bats, Kenya. *Emerging Infectious Diseases*. 16(12):1875–1881.

Lei BR, Olival KJ. 2014. Contrasting patterns in mammal–bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. *PLoS Neglected Tropical Diseases.* 8(3):e2738.

Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, Parola P. 2016. Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. *Parasites & Vectors.* 9:2.

Lin J-W, Hsu Y-M, Chomel BB, Lin L-K, Pei J-C, Wu S-H, Chang C-C. 2012. Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*. *Veterinary Microbiology*. 156:119–126.

Loftis AD, Gill JS, Schriefer ME, Levin ML, Eremeeva ME, Gilchrist MJ, Dasch GA. 2005. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). *Journal of Medical Entomology.* 42(3):473–480.

Mannerings AO, Osikowicz LM, Restif O, Nyarko E, Suu-Ire R, Cunningham AA, Wood JL, Kosoy MY. 2016. Exposure to bat-associated bartonella spp. among humans and other animals, Ghana. *Emerging Infectious Diseases*. 22(5):922–924.

McQuiston J. 2016. Infectious diseases related to travel. In: *CDC Health Information for International Travel 2016*. GW Brunette (ed.). Centers for Disease Control and Prevention: Atlanta, GA.

Morse SF, Olival KJ, Kosoy M, Billeter S, Patterson BD, Dick CW, Dittmar K. 2012. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). *Infection, Genetics and Evolution.* 12:1717–1723.

Olival KJ, Dittmar K, Bai Y, Rostal MK, Lei BR, Daszak P, Kosoy M. 2015. *Bartonella* spp. in a Puerto Rican bat community. *Journal of Wildlife Diseases*. 51(1):274–278.

Piksa K, Górz A, Nowak-Chmura M, Siuda K. 2014. The patterns of seasonal activity of *Ixodes vespertilionis* (Acari: Ixodidae) on *Rhinolophus hipposideros* in nursery colonies. *Ticks and Tick-borne Diseases.* 5:69–74.

Piksa K, Stańczak J, Biernat B, Górz A, Nowak-Chmura M, Siuda K. 2016. Detection of *Borrelia burgdorferi sensu lato* and spotted fever group rickettsiae in hard ticks (Acari, Ixodidae) parasitizing bats in Poland. *Parasitology Research*. DOI 10.1007/s00436-016-4936-2.

Reeves WK, Rogers TE, Durden LA, Dasch GA. 2007. Association of *Bartonella* with the fleas (Siphonaptera) of rodents and bats using molecular techniques. *Journal of Vector Ecology.* 32(1):118–122.

Reeves WK, Streicker DG, Loftis AD, Dasch GA. 2006. Serologic survey of *Eptesicus fuscus* from Georgia, U.S.A. for *Rickettsia* and *Borrelia* and laboratory transmission of a *Rickettsia* by bat ticks. *Journal of Vector Ecology*. 31(2):386–389.

Schaer J, Reeder DM, Vodzak ME, Olival KJ, Weber N, Mayer F, Matuschewski K, Perkins SL. 2015. *Nycteria* parasites of Afrotropical insectivorous bats. *International Journal for Parasitology.* 45:375–384.

Schwan TG, Raffel SJ, Schrumpf ME, Gill JS, Piesman J. 2009. Characterization of a novel relapsing fever spirochete in the midgut, coxal fluid, and salivary glands of the bat tick *Carios kelleyi*. *Vector-Borne and Zoonotic Diseases.* 9(6):643–647.

Socolovschi C, Kernif T, Raoult D, Parola P. 2012. *Borrelia*, *Rickettsia*, and *Ehrlichia* species in bat ticks, France, 2010. *Emerging Infectious Diseases.* 18(12):1966–1975.

Steinlein DB, Durden LA, Cannon WL. 2001. Tick (Acari) infestations of bats in New Mexico. *Journal of Medical Entomology.* 38(4):609–611.

Tahir D, Socolovschi C, Marié J-L, Ganay G, Berenger J-M, Bompar J-M, Blanchet D, Cheuret M, Mediannikov O, Raoult D, Davoust B, Parola P. 2016. New Rickettsia species in soft ticks *Ornithodoros hasei* collected from bats in French Guiana. *Ticks and Tick-Borne Diseases.* 7(6):1089–1096.

Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT. 2014. Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis*. *Emerging Infectious Diseases.* 20(6):960–967.

Walker DH. 1996. Rickettsiae. In: *Medical Microbiology*, 4th edition. S Baron (ed.). University of Texas Medical Branch at Galveston: Galveston, TX, Chapter 38.

Wilkinson DA, Duron O, Cordonin C, Gomard Y, Ramasindrazana B, Mavingui P, Goodman SM, Tortosa P. 2016. The bacteriome of bat flies (Nycteribiidae) from the Malagasy region: a community shaped by host ecology, bacterial transmission mode, and host-vector specificity. *Applied Environmental Microbiology.* 82(6):1778–1788.

10

OTHER BACTERIA AND BATS

10.1 INTRODUCTION

The nutritionally rich intestinal microbiome of animals, including humans and bats, is densely populated and contains both beneficial and pathogenic bacteria as well as opportunistic bacteria. See Table 10.1 for a list of various bacteria with reported association to bats. Trillions of gut bacteria are beneficial to the host species, aiding in nutrient metabolism and producing vitamins and additional molecules that are needed by the host (Cummings & Macfarlane 1997). Further roles of these bacteria include assisting in food digestion and modulation of host metabolism and immune functioning. They also are active in host evolution (Banskar *et al*. 2016). The importance of normal bacterial flora may be seen by the consequences associated with the loss of these bacteria, such as disruption of digestive functioning and overgrowth of yeast in the oral cavity (thrush) which may be resolved by ingesting probiotics (bacteria).

Many bacteria, however, are pathogenic and may cause severe disease or death. Some of the pathogens are able to jump the species barrier and infect a wide range of vertebrates and invertebrates. The development of antibiotic resistance to pathogenic bacteria is a subject of increasing concern for humans and domestic animals as well as bats. Antibiotic resistance is highly prevalent in *E. coli* taken from bats inhabiting both urban and wilderness areas of Mexico. *E. coli* from 46% of tested bats were resistant to ampicillin and 100% of these bacteria were resistant to streptomycin. Antibiotic resistance of *E. coli* in bats has been reported in regions as distant as Trinidad and Australia (Mühldorfer *et al*. 2011b). The practice of bat guano collection directly exposes people to bat intestinal bacteria, including *E. coli*, and may lead to zoonotic transmission. This

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TABLE 10.1 Various bacteria associated with bats

Bat family	Bat common name	Bat species	Bacteria
Phyllostomidae	Gnome fruit-eating bat	*Artibeus gnoma*	*Leptospira* sp.
Phyllostomidae	Great fruit-eating bat	*Artibeus literalis*	*Waddlia cocoyoc*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Salmonella* serotype *sandiego*
Phyllostomidae	Dark fruit-eating bat	*Artibeus obscurus*	*Leptospira* sp.
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	*Leptospira* sp.
Phyllostomidae	Silky short-tailed bat	*Carollia brevicauda*	*Leptospira* sp.
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Leptospira* sp.
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	*Salmonella enterica* subspecies Enterica
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	*Salmonella enteritidis*
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	*Koserella trabulsii*
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	*Kluyvera* sp.
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	*Serratia marcescens*
Vespertilionidae	Little fruit-tailed bat	*Chaerephon pumila*	atypical *Hafnia alvei*
Vespertilionidae	Aldabra free-tailed bat	*Chaerephon pusillus*	*Leptospira* sp.
Pteropodidae	Short-nosed fruit bat	*Cynopterus brachyotis*	*Salmonella* sp.
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Bacillus cereus*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Bacillus thuringiensis*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterobacter aerogenes*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterobacter amnigenus*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterobacter cancerogenus*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterobacter cloacae*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterobacter hormaechei*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Enterococcus faecalis*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Escherichia coli*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Escherichia hermannii*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Klebsiella oxytoca*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Klebsiella pneumonia*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Pantoea agglomerans*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Pseudomonas aeruginosa*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis brachyotis*	*Serratia marcescens*
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis javanicus*	*Citrobacter* sp.
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis javanicus*	*Escherichia coli*
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Citrobacter* sp.

Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Enterobacter* sp.
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Escherichia coli*
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Klebsiella* sp.
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Pseudomonas* sp.
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx angulatus*	*Serratia* sp.
Pteropodidae	Indonesian short-nosed fruit bat	*Cynopterus tittaecheilus tittaecheilus*	*Citrobacter* sp.
Pteropodidae	Indonesian short-nosed fruit bat	*Cynopterus tittaecheilus tittaecheilus*	*Enterobacter* sp.
Pteropodidae	Indonesian short-nosed fruit bat	*Cynopterus tittaecheilus tittaecheilus*	*Escherichia coli*
Pteropodidae	Indonesian short-nosed fruit bat	*Cynopterus tittaecheilus tittaecheilus*	*Klebsiella* sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Aeromonas hydrophila*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Enterobacter*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Escherichia coli*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Leptopira* sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Proteus* species
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Staphylococcus* sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Salmonella enterica* Arizona
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Salmonella typhimurium*
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	*Leptopira* sp.
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Leptospira borgpetersenii*
Pteropodidae	Straw-colored fruit bat	*Eidolon helvum*	*Leptospira kirschneri*
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	*Waddlia malaysiensis*
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	*Salmonella* sp.
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Pseudomonas* sp.
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	*Pasteurella* sp.
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	*Vespertiliibacter pulmonis*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Leptospira* sp.
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Salmonella typhimurium*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Salmonella saint paul*
Hipposideridae	Diadem roundleaf bat	*Hipposideros diadema*	*Salmonella* sp.
Phyllostomidae	Thomas's nectar bat	*Lonchophylla thomasi*	*Leptospira* sp.
Phyllostomidae	Stripped hairy-nosed bat	*Mimon crenulatum*	*Leptospira* sp.
Miniopteridae	Little long-fingered bat	*Miniopterus australis*	*Salmonella* sp.
Miniopteridae	Glen's long-fingered bat	*Miniopterus gleni*	*Leptospira* sp.
Miniopteridae	None	*Miniopterus griffithsi*	*Leptospira* sp.

(*Continued*)

TABLE 10.1 (Continued)

Bat family	Bat common name	Bat species	Bacteria
Miniopteridae	None	*Miniopterus griveaudi*	*Leptospira* sp.
Miniopteridae	None	*Miniopterus mahafaliensis*	*Leptospira* sp.
Miniopteridae	Schreibers' bat	*Miniopterus schreibersii*	*Mycoplasma* sp.
Miniopteridae	Schreibers' bat	*Miniopterus schreibersii*	*Candidatus Mycoplasma hemominiopterus*
Miniopteridae	Schreibers' bat	*Miniopterus schreibersii*	*Salmonella* sp.
Molossidae	Black mastiff bat	*Molossus ater*	*Salmonella* Group I
Molossidae	None	*Molossus major*	*Salmonella* serotype Caracas
Molossidae	Bonda's mastiff bat	*Molossus bondae*	*Shigella boydii-2*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Salmonella* serotype *anatum*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Salmonella* serotype *blockley*
Molossidae	None	*Mormopterus francoismoutoui*	*Leptospira* sp.
Molossidae	Peter's wrinkle-tipped bat	*Mormopterus jugularis*	*Leptospira* sp.
Vespertilionidae	Long-eared bat	*Myotis capaccinii*	*Mycoplasma* sp.
Vespertilionidae	Daubenton's bat	*Myotis daubentonii*	*Cedecea davisae*
Vespertilionidae	Malagasy mouse-eared bat	*Myotis goudoti*	*Leptospira* sp.
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Candidatus Mycoplasma hemominiopterus*
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Mycoplasma haemomuris*
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Pseudomonas* sp.
Vespertilionidae	Pallid large-footed myotis	*Myotis macrotarsus*	*Salmonella* sp.
Vespertilionidae	Greater mouse-eared bat	*Myotis myotis*	*Yersinia pseudotuberculosis*
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Pasteurella multocida*
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Enterobacteriaceae*
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Enterococcus faecalis*
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Clostridium sordellii*
Vespertilionidae	Nepalese whiskered bat	*Myotis muricola muricola*	*Morganella* sp.
Vespertilionidae	Nepalese whiskered bat	*Myotis muricola muricola*	*Proteus/Providencia* sp.
Vespertilionidae	Natterer's bat	*Myotis nattereri*	*Enterococcus faecalis*
Vespertilionidae	Ripian bat	*Myotis riparius*	*Leptospira* sp.
Noctionidae	Greater bulldog bat	*Noctilio leporinus*	*Salmonella* serotypes Rubislaw and Molade
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Clostridium perfringens*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Clostridium sordellii*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Escherichia coli*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Salmonella* sp.

Vespertilionidae	Common noctule	*Nyctalus noctula*	*Staphylococcus aureus*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Vespertiliibacter pulmonis*
Vespertilionidae	Malagasy giant mastiff bat	*Otomops madagascariensis*	*Leptospira* sp.
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	*Leptospira* sp.
Vespertilionidae	Himalayan pipistrelle	*Pipistrellus javanicus*	*Salmonella* sp.
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	*Pasteurella multocida*
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	*Staphylococcus aureus*
Vespertilionidae	Nathusius' pipistrelle	*Pipistrellus nathusii*	*Enterobacteriaceae*
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Yersinia enterocolitica*
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Pasterela multocida* sp. septica
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Microbacter pulmonis*
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Enterococcus faecalis*
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Bacillus cereus*
Vespertilionidae	Soprano pipistrelle	*Pipistrellus pygmaeus*	*Mycoplasma* sp.
Vespertilionidae	Pipestrelles	*Pipistrellus* sp.	*Neorickettsia risticii*
Phyllostomidae	Heller's broad-nosed bat	*Platyrhinus helleri*	*Leptospira* sp.
Phyllostomidae	White-lined broad-nosed bat	*Platyrrhinus lineatus*	*Leptospira* sp.
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	*Pasteurella multocida*
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	*Enterobacteriaceae*
Molossidae	Big-crested mastiff bat	*Promops centralis*	*Leptospira* sp.
Molossidae	Brown mastiff bat	*Promops nasutus*	*Leptospira* sp.
Pteropodidae	Greater musky fruit bat	*Ptenochirus jagori*	*Salmonella* sp.
Pteropodidae	Black flying fox	*Pteropus alecto*	*Leptospira* sp.
Pteropodidae	Spectacled flying fox	*Pteropus conspicillatus*	*Leptospira* sp.
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Citrobacter freundii*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Clostridium septicum*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Klebsiella oxytoca*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Micrococcus* sp.
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Proteus mirabilis*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Proteus vulgaris*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Salmonella* Virchow
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Serratia liquefaciens*
Pteropodidae	Indian flying fox	*Pteropus giganteus*	*Staphylococcus* sp.
Pteropodidae	Island flying fox	*Pteropus hypomelanus*	*Clostridium septicum*
Pteropodidae	Island flying fox	*Pteropus hypomelanus*	*Morganella morganii*

(*Continued*)

TABLE 10.1 (Continued)

Bat family	Bat common name	Bat species	Bacteria
Pteropodidae	Island flying fox	*Pteropus hypomelanus*	*Stapylococcus aureus*
Pteropodidae	Island flying foxes	*Pteropus hypomelanus*	*Pasteurella*-like bacteria
Pteropodidae	Grey-headed flying fox	*Pteropus poliocephalus*	*Leptospira* sp.
Pteropodidae	Little golden mantled flying fox	*Pteropus pumilus*	*Cornebacterium* sp.
Pteropodidae	Little golden mantled flying fox	*Pteropus pumilus*	*Pasteurella*-like bacteria
Pteropodidae	Little golden mantled flying fox	*Pteropus pumilus*	*Proteus* sp.
Pteropodidae	Little golden mantled flying fox	*Pteropus pumilus*	*Staphylococcus* sp.
Pteropodidae	Rodrigues fruit bat	*Pteropus rodricensis*	*Pasteurella*-like bacteria
Pteropodidae	Madagascar flying fox	*Pteropus rufus*	*Salmonella enterica* Typhi
Pteropodidae	Little red flying fox	*Pteropus scapulatus*	*Leptospira* sp.
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Citrobacter freundii*
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Klebsiella oxytoca*
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Pasteurella*-like bacteria
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Proteus mirabilis*
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Proteus vulgaris*
Pteropodidae	Malaysian flying fox	*Pteropus vampyrus*	*Serratia liquefaciens*
Phyllostomidae	Dwarf little fruit bat	*Rhinophylla pumilio*	*Leptospira* sp.
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	*Yersinia pseudotuberculosis*
Pteropodidae	Geoffroy's rousette	*Rousettus amplexicaudatus*	*Salmonella* sp.
Pteropodidae	Comoro rousette	*Rousettus obliviosus*	*Leptospira borgpetersenii*
Pteropodidae	Comoro rousette	*Rousettus obliviosus*	*Leptospira interrogans*
Phyllostomidae	Yellow-shouldered bat	*Sturnira lilium*	*Leptospira* sp.
Phyllostomidae	Yellow-shouldered bat	*Sturnira lilium*	*Salmonella llandoff*
Phyllostomidae	Tilda yellow-shouldered bat	*Sturnira tildae*	*Leptospira* sp.
Vespertilionidae	Asiatic lesser yellow house bat	*Scotophilus kuhlii*	*Salmonella* sp.
Pteropodidae	Long-tongued fruit bat	*Sycnycteris crassa*	*Leptospira* sp.
Rhinolophidae	Trouessart's trident bat	*Triaenops furculus*	*Leptospira* sp.
Rhinolophidae	Rufous trident bat	*Triaenops menamena*	*Leptospira* sp.
Rhinolophidae	Persian trident bat	*Triaenops persicus*	*Leptospira* sp.
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	*Leptospira* sp.
Vespertilionidae	Parti-colored bat	*Vespertilio murinus*	*Pasteurella multocida*

may be a health hazard if the strain of *E. coli* is O157:H7 or another Shiga-toxin producing strain, since some of the severe manifestations, such as hemolytic uremic syndrome and hemorrhagic colitis, may be fatal. Even though bat guano might contain highly pathogenic *E. coli*, sheep and cattle feces or undercooked beef are much more likely to serve as sources of human infection since a study in the UK found that more than 10% of seemingly healthy cattle were infected with this bacterial strain (reviewed by Beltz 2011). More worrisome, methicillin-resistant *Staphylococcus aureus* (MRSA) was also detected in a German bat (Mühldorfer *et al*. 2011b). MRSA is resistant to many of the most commonly used antibiotics and in the past few decades has spread rapidly in humans throughout the world.

10.2 *LEPTOSPIRA*

Leptospirosis is a major re-emerging bacterial threat throughout the world, affecting humans and domestic and wild animals. It is the most common zoonotic infection in the world at present, especially in tropical regions. *Leptospira interrogans* is the species which most frequently infects humans, with murid rats serving as its vector. It is estimated that more than 500 000 cases of severe leptospirosis occur each year in humans and the mortality rate is greater than 10%. Additional asymptomatic and subclinical infections are common. Interestingly, a study of DNA extracted from kidneys of 98 big brown bats (*Eptesicus fuscus*) in the Central Plains area of the US did not detect any pathogenic leptospires, decreasing the possible role of these bats from serving as a viral reservoir for humans or dogs in the area (Harkin 2014).

Leptospirosis is caused by pathogenic members of *Leptospira*. The genus contains at least 22 species and a number of serovars. The species are divided into 10 pathogenic species (including at least 200 pathogenic serovars), 5 species of intermediate pathology, and 7 saprophytic (nonparasitic) species. Leptospirosis begins with acute, febrile disease with severe malaise, muscle pain, and conjunctival suffusion. In some cases, it may evolve into Weil's disease, a severe hemorrhagic illness that may be confused with a viral hemorrhagic fever. It might also become fatal if there is involvement of the hepatic, renal, pulmonary, or central nervous systems (reviewed by Cox *et al*. 2005 and Vashi *et al*. 2009). The causative bacteria are Gram-negative, obligate anaerobic spirochetes. These extracellular bacteria inhabit the renal tubules of the kidneys and are excreted in the urine. They survive up to 6 weeks in soil or water. Infection occurs when mucous membranes or abraded skin is exposed to infected urine or urine-contaminated water or soil (reviewed by Vashi *et al*. 2009).

10.2.1 *Leptospira* in South America

Bunnell *et al*. (2000) found genetic evidence of renal infection of 35% of tested bats with pathogenic *Leptospira* in the Peruvian Amazon Basin ($n = 20$). A more recent and much larger study of *Leptospira* in bats from the Peruvian Amazon ($n = 589$) reported that 3.4% of the bats' kidneys were positive for *Leptospira* either by PCR or by culture (Matthias *et al*. 2005). The species of leptospires detected in this study were diverse and included *L. interrogans, Leptospira kirschneri, Leptospira borgpetersenii, Leptospira fainei*, plus two novel species. All of the infected bat species in this study were of the

family Phyllostomidae with the exception of one *Myotis riparius* (vespertilionid) and one *Promops nasutus* (molosid). Prevalence of *Leptospira* infection in adult bats was four times that in non-sexually mature animals. Additionally, the rate of infection of bats from mature forests was higher than the combined rate of agricultural land and secondary growth forests. Bats, via urine, may aid in transmission of *Leptospira* to humans, however, both dogs and rats have a high rate of infection with bacterial serovars known to infect humans as well and so may also have important roles in human infection. Interestingly, some bats were infected by *L. interrogans* serovar Icterohemorrhagiae, which generally inhabit peridomestic rats. This suggests the possibility of a rodent–bat infection cycle.

The biodiversity of *Leptospira* species is lower in the urban centers of Brazil than in the rural areas of the Amazon Basin. In order to determine the potential importance of bats as vectors of pathogenic *Leptospira* species, PCR was used to detect DNA of pathogenic spirochetes in the large urban center of São Paulo, which has the highest rate of human infection in Brazil. Out of a sample of 343 bats, only 6 were found to carry *Leptospira* DNA in their kidneys and none of the bats were seropositive (Bessa *et al*. 2010). Over 150 of both insectivorous and frugivorous or nectivorous bats were tested. Four of the PCR-positive bats were nectivorous *Glossophaga soricina* and two were frugivorous *Platyrrhinus lineatus*. Both of these species preferentially inhabit human structures with little human activity. This study suggests that in São Paulo, and perhaps other major urban regions, bats are not important in transmission of *Leptospira* to humans, but rather that urban rats and dogs found in the unsanitary slums may have a far greater role in transmission (Bessa *et al*. 2010).

10.2.2 *Leptospira* in Africa

A recent study in Tanzania found a high prevalence of leptospirosis in individual bats (19.4%) and even higher within bat colonies (27.3%). *Leptospira* serovar Sokoine was the most prevalent (19.4%), followed by serovars Kenya (2.8%) and Lora (2.8%) (Mgode *et al*. 2014). In a group of bats migrating from Democratic Republic of Congo to Zambia, molecular studies of bacterial DNA in the kidneys of the straw-colored fruit bat (*Eidolon helvum*) detected the flagellin B gene from pathogenic *Leptospira* species in nearly 15% of the animals ($n=529$). Phylogenetic analysis of samples isolated from 70 bats demonstrated that 12 sample fragments grouped with *L. borgpetersenii* and *L. kirschneri* and the remaining fragments were novel (Ogawa *et al*. 2015). When a subset of 16S ribosomal RNA genes were compared with those previously reported, all 27 fragments clustered into a pathogenic group. Bat rRNA are genetically related and no regional variations have been found in phylogenetic analysis of sequences from various kinds of hosts. This suggests that *Leptospira* are evolving uniquely in at least these bats (Ogawa *et al*. 2015). Lei and Olival's study of both Old and New World bats, however, indicates that *Leptospira* has no evolutionary congruence with its bat hosts and undergoes a high number of host switches (Lei & Olival 2014).

10.2.3 *Leptospira* in islands of the Indian Ocean

Leptospirosis in humans poses a major public health concern in certain islands in the southwestern Indian Ocean, especially La Réunion, Mayotte, and the Seychelles. However, it has not been detected in humans in the nearby islands of Madagascar and

Union of the Comoros. Pathogenic *Leptospira* species recently found in small mammals introduced to Madagascar may serve as a harbinger of human infection. Bats from these latter islands were tested for the presence of *Leptospira* DNA in pooled kidneys, spleen, and lung. Of the twelve Madagascar bat species tested, eleven were positive for *Leptospira* DNA: *Mormopterus jugularis, Otomops madagascariensis, Triaenops furculus, Triaenops menamena, Miniopterus gleni, Miniopterus griffithsi, Miniopterus mahafaliensis*, and *Myotis goudoti* as were all three species of bats from the Union of the Comoros: *Rousettus obliviosus, Chaerephon pusillus*, and *Miniopterus griveaudi* (Lagadec *et al*. 2012). The bacterial species identified were closely related to *L. borgpetersenii* and *L. interrogans*. Both of these *Leptospira* species were identified from *R. obliviosus* bats sharing a cave in the Union of the Comoros. The *L. borgpetersenii* sequence was closely related to that from *O. madagascariensis* bats from Madagascar.

A separate study in Réunion Island examined bat population dynamics in relation to *Leptospira* shedding in a *Mormopterus francoismoutoui* maternity colony (Dietrich *et al*. 2015). Infection with *Leptospira* and spirochete shedding in the urine peaked late during pregnancy, with a 45% infection rate in pregnant females. This phenomenon may be due to altered hormonal levels associated with suppressed immunity common in many animals during pregnancy. Interestingly, during parturition, the infection rates and intensity of bacterial excretion was decreased to as little as 6% (±6%), perhaps due to passively acquired antibodies across the placenta or in the mother's milk. A second infection peak of 62% then occurred 2 months after the highly synchronized birthing period and might be due to infection of the large numbers of nonimmune young bats. Similar infection dynamics were found in bat paramyxoviruses, also included in the study.

A 2014 study of *Leptospira* evolution within small mammals from Madagascar examined diversity and genetic relationships of these bacteria in endemic bat populations (Dietrich *et al*. 2014). Other factors contributing to bacterial diversity include geographical isolation, colonization, and evolutionary cospeciation. Madagascar is one of the most important "hot spots" for biological diversity and the range of large numbers of endemic species. Only five nonendemic small mammals are known to have been introduced to the island, including *Rattus rattus*, the major reservoir host of leptospiridians. Due to the supposition that the bat–rat transmission cycle might be a driving factor in rat infection, the presence of infection of endemic Malagasy bats from the Miniopteridae and Vespertilionidae families was examined at the molecular level (Dietrich *et al*. 2014). Recent studies using PCR have detected pathogenic *Leptospira* in both introduced and endemic animal species, including bats. Of note, endemic and introduced small mammals appear to carry different *Leptospira* species.

10.2.4 *Leptospira* in Australia

A real-time PCR study of Australian flying foxes (*Pteropus* species) detected pathogenic leptospiral DNA in 11% of kidney ($n = 173$) and 39% of urine samples ($n = 46$) of four species of flying fox: the spectacled flying fox (*Pteropus conspicillatus)*, black flying fox *(Pteropus alecto)*, grey-headed flying fox *(Pteropus poliocephalus)*, and little red flying fox (*Pteropus scapulatus)*. All of the tested flying fox species had leptospiral DNA in their kidneys and urine, with no significant differences between bat species (Cox *et al*. 2005). These findings indicate that Australian flying foxes shed leptospires into the environment.

A separate study in Australian flying foxes reported antibodies to seven *Leptospira* serovars in 28% of the bats' sera ($n=271$). One of these serovars, *L. interrogans* serovar cynopteri, had not been previously found in Australia and may be an emergent serovar. The *L. kirschneri* serovar australis was most frequently detected in this study (60.2% infection rate) (Smythe *et al*. 2002). The other serovars identified in these bats were hardjo, bulgarica, tarassovi, pomona, and canicola.

Since rodents are believed to play a major role in the transmission of leptospires to humans, a year-long study was performed to determine whether proximity to Australian fruit bat colonies, specifically *P. conspicillatus*, was associated with the prevalence of leptospiral infection of the rodent fawn-footed melomys (*Melomys cervinipes*) ($n=213$). Interestingly, the numbers of this rodent which inhabited areas close to bat colonies was over four times less than areas at least 2000 m away from a bat colony, perhaps due to damage inflicted by bat colonies to the forest understory that is the rodents' habitat as well as the presence of bats enticing predators into the area. The prevalence of leptospiral carriage in the rodents' kidneys, however, was 100% close to bat colonies, compared with 3.6% in areas further from the bats (Tulsiani *et al*. 2011).

10.3 *YERSINIA*

Yersinia are small, nonmotile, Gram-negative coccobacilli with strong bipolar staining. Mühldorfer *et al*. (2010) cultured 25 bacterial genera from 16 species of insectivorous bats throughout Germany. These included the human pathogens, *Yersinia pseudotuberculosis*, associated with severe diarrhea and local abscesses, and *Yersinia enterocolitica*, associated with severe enterocolitis in humans. *Y. pseudotuberculosis* was isolated from lung, heart, and kidney cultures of the greater mouse-eared bat (*Myotis myotis*). The bat had a severely enlarged liver and marked hemoperitoneum. Microscopic examination showed multifocal severe necrotizing hepatitis, splenitis, and interstitial pneumonia. *Y. enterocolitica* was cultured from the spleen and intestine of common pipistrelles (*Pipistrellus pipistrellus*) with subclinical infection.

Y. pseudotuberculosis killed seven bats from a closed colony of Egyptian fruit bats (*Rousettus aegyptiacus*) in a New York zoo. Two bats had acute disease with sepsis, multiorgan failure, and rapid death. The other five bats had chronic and debilitating disease. Upon initial observation, 41.7% of bats ($n=12$) had visceral abscesses of the liver, spleen, kidneys, and lungs characteristic of pseudotuberculosis. Additionally, 70% of the colony members ($n=115$) had symptoms suggestive of *Y. pseudotuberculosis* infection: mesenteric lymphadenopathy, hepatic abscesses, or splenomegaly (Childs-Sanford *et al*. 2009). Gram-negative coccobacilli were present in some of the necrotizing lesions. *Y. pseudotuberculosis* was cultured from four of the animals examined. These bacteria are primarily transmitted via the fecal–oral route. Accordingly, population density and mortality rates were correlated and the mortality rate of subadult bats increased greatly during the outbreak at the time of highest population density. Stress, in addition to the resulting immunosuppression due to severe overcrowding, might predispose bat colonies to morbidity and mortality (Childs-Sanford *et al*. 2009). Since birds and rodents are believed to be the primary reservoirs for *Y. pseudotuberculosis*, contact with wild rodents may have led to this outbreak in bats, especially due to a coinciding rodent infestation of the facility.

10.4 *PASTEURELLA*

Pasteurella species are aerobic, Gram-negative bacilli. They are nonpathogenic for cats and dogs and are part of their normal nasopharyngeal flora. *Pasteurella*, however, may cause life-threatening pneumonia in other domestic animals, such as cattle, sheep, and birds. Infected humans may develop abscesses of the extremities or face after been bitten by infected cats or dogs (Collins 1996). *Pasteurella* species, including *P. multocida*, *P. pneumotropica*, and *Pasteurella* species B, cause several types of localized or systemic infections in European bats and caused severe pneumonia and subcutaneous abscesses in a captive flying fox. Carnivores harbor pathogenic *Pasteurella* species as commensals in their oropharyngeal cavity and can transfer them via bites. Most bats dying from *Pasteurella* infections bore wounds similar to those acquired by cat bites (reviewed by Mühldorfer *et al*. 2011b).

Aerobic, Gram-negative bacilli were isolated from lung tissue or tracheal wash fluid of captive Wahlberg's epauleted fruit bats (*Epomophorus wahlbergi*) and a captive Malaysian flying fox (*Pteropus vampyrus*) in the US (Helmick *et al*. 2004). These animals had severe unilateral pneumonia prior to death. The bacteria were also found in pharyngeal swabs from island flying foxes (*Pteropus hypomelanus*) and a Rodrigues fruit bat (*Pteropus rodricensis*) and in subcutaneous abscesses from two captive little golden mantled flying foxes (*Pteropus pumilus*). Biochemical testing and fatty acid profiles suggest that the organism is a novel species of either *Pasteurella* or a *Pasteurella*-like microbe.

Since *Pasteurella* are an important cause of fatal infections in free-ranging bats, Mühldorfer *et al*. (2011a) examined the diversity of *Pasteurella* strains of diseased free-range bats ($n = 394$) over a 6-year period in three distinct regions of Germany. Using molecular techniques, 81 strains of *Pasteurella* were found in vespertilionid bats. The infected bat species are as follows: *Pipistrellus* species – *P. pipistrellus, P. pygmaeus, P. kuhlii*, and *Pipistrellus nathusii; Plecotus auritus; Vespertilio murinus; Myotis mystacinus*; and *Eptesicus serotinus*. Infection was linked to the presence of pneumonia, severe organ necrosis, and systemic infection. *P. multocida, P. septica*, and *P. multocida* species Multocida composed the majority of *Pasteurella* species found in the bats (85%), but a few infections with *Pasteurella* species B were also present (Mühldorfer *et al*. 2011a). Two separate studies of deceased free-ranging European bats reported some similar findings and many of the fatalities due to pasteurellosis also had traumatic injuries (fractures or wing lacerations), 50–65% of which were directly attributed to cat predation (Simpson 2000). In this study, *P. multocida*-induced septicemia killed 22% of the bats, all of which had been bitten by cats. Since cats' oral mucosa typically contains pathogenic *Pasteurella* strains, cat attacks might subsequently lead to pasteurellosis in the bats and death from septicemia.

Five strains of novel new genus of *Pasteurellaceae* bacteria were detected and molecularly characterized from three species of *Vespertilionidae* bats: three *Nyctalus noctula*, one *P. pipistrellus*, and one *E. serotinus* (Mühldorfer *et al*. 2014). This novel genus is differentiated from other *Pasteurellaceae* by its requirement for supplemental NAD for growth and by its G+C DNA content. *Vespertiliibacter pulmonis* was the proposed name for these Gram-negative coccobacilli.

10.5 *MYCOPLASMA*

Hemotropic mycoplasmas are emerging or re-emerging pathogens responsible for serious health problems in humans, some wild mammals, and livestock throughout the world. These bacteria, which lack cell walls, dwell on the external surface of erythrocytes and are causative agents of infectious anemia, particularly in the presence of a coinfection with a more virulent pathogen in humans. A British patient, however, was reported to have developed severe hemolytic anemia caused by "*Candidatus* Mycoplasma hemohominis" in the absence of another pathogen (Steer *et al*. 2011). Pathology ranges from asymptomatic to life-threatening hemolytic anemia, subtle chronic anemia, and infertility. Mycoplasma may also serve as cofactors in the progression of retroviral or immune-mediated diseases and some cancers (reviewed in Mascarelli 2014; Millán 2015).

In a study of dead little brown bats (*Myotis lucifugus*) from Appalachian US, 47% of the hemotropic bats were infected by mycoplasmas whether with (49%; $n=53$) or without white-nose syndrome (40%; $n=15$). These bacteria appear to belong to a novel hemotropic *Mycoplasma* species that has 91.8% sequence homology with *Mycoplasma haemomuris* (Mascarelli *et al*. 2014). The presence of two sequences of 16S rRNA from hemoplasma was detected in blood samples from 97% of 31 bats from two caves and a mine in a region of northeastern Spain. Schreibers' bats (*Miniopterus schreibersii;* $n=22$) and a long-eared bat (*Myotis capaccinii*) had DNA sequences from one group of seven closely related sequences having 97% identity with "*Ca. M. hemohominis*" in a phylogenetic branch containing both bat and human sequences. The second group of mycoplasma DNA sequences, found in six *M. schreibersii*, is postulated to belong to a potentially new species tentatively named "*Candidatus Mycoplasma hemominiopterus*". This sequence was 91% identical to that from the above-mentioned hemoplasma infecting *M. lucifugus* (Millán *et al*. 2015). *M. schreibersii* are very gregarious, colonial bats roosting in caves and mines that benefit energetically by either direct contact with other species or close to large aggregations. This roosting behavior may contribute to intra- and interspecies spread of bacteria.

10.6 *WADDLIA*

A novel species of chlamydia-like obligate intracellular bacteria was isolated from the urine of 3.5% ($n=206$) of nectivorous lesser dawn bats (*Eonycteris spelaea*) from peninsular Malaysia. Upon growth in human, simian, or rodent cell lines, the bacteria produce large membrane-bound inclusions containing reticulate and elementary bodies. The inclusions were surrounded by mitochondria and were positive for periodic acid-Schiff stain but not by antibodies to *Chlamydia trachomatis* major outer membrane protein. The bacteria could not be cultured on blood or chocolate agar, aerobically or anaerobically, for up to 7 days. Chalmydiales have recently been proposed to be split into four families, including *Chlamydiaceae* and *Waddliaceae*. Analysis of 16S rRNA indicates the reported bacteria are most closely related to a species, *Waddlia chondrophila*, linked to bovine abortions and human miscarriages. Since the 16S and 23S rRNA gene signatures were 91% identical, this is postulated to be a novel species tentatively named *Waddlia malaysiensis* (Chua *et al*. 2005).

A second species of *Waddlia* was recently isolated from an adult great fruit-eating bat (*Artibeus literalis*), common in the tropical Americas. The affected animal (negative for

the rabies virus) demonstrated emaciation, restlessness, depression, and loss of flight activity. Prior to death, regions of pallor not due to fungal infection appeared on its wings. These regions contained multinuclear infiltrates. Experimental infection of other bats resulted in mild to severe multifocal interstitial pneumonia and severe diffuse lymphoid hyperplasia in the spleen. Intracytoplasmic vacuoles appeared during culture in Vero cells. Sequence analyses showed the bacteria are closely related to *Waddlia* species and have tentatively been named *Waddlia cocoyoc*, due to its site of isolation in Cocoyoc, Mexico (Pierlé *et al*. 2015).

10.7 RICKETTSIA AND SIMILAR BACTERIA

Antibodies against *Orienta tsutsugamushi* (the causative agent of scrub typhus in humans), *Borrelia* species (*Borrelia hermsii*; relapsing fever group), and *Rickettsia* species (*Rickettsia conorii, Rickettsia rickettsii, Rickettsia parkeri, Rickettsia amblyommii*, and *Rickettsia rhipicephali*; spotted fever group) are present in bat serum from widely separated areas – Korea, Brazil, and the southern US. Most of the bats are members of the Vespertilionidae or Molossidae families (reviewed by Mühldorfer 2013).

10.8 BAT GASTROINTESTINAL TRACT BACTERIA

Gut bacteria in bats are influenced by the hosts and therefore may promote a greater understanding about feeding and diet of the host bats. Higher bacterial diversity is generally associated with good health while lesser diversity is often associated with diseased animals (Scott *et al*. 2015). In a quantitative study of bacterial composition of three neotropical bat species, the following average bacterial populations were as follows: *Molossus major*, $10^{4.8}$ bacteria per intestinal contents; *Chilonycteris rubiginosa*, $10^{3.9}$; and *Carollia perspicillata*, $10^{3.3}$, with the *Klebsiella–Aerobacter–Serratia* group being detected most frequently, followed by enterococci and *Proteus* species. Other bacterial species, found less commonly in these bats, include *Escherichia, Alcaligenes*, and *Bacteroides* species. Mice, however, averaged $10^{9.7}$ bacteria per intestinal contents (Klite 1965). Several factors may contribute to the much smaller amounts of bacteria in the intestines of bats compared to mice. The length of these bats' intestines are one-third to one-fifth that of a mouse of comparable weight. Bats lack the large intestine, cecum, and appendix. The transit time through the short bat intestine is 15 minutes and bats defecate an average of 60 times daily. While contents of intestinal pH for mice and bats ranged between 6.0 and 7.0, the intestinal pH of mice was 0.2 to 0.5 units higher than that of bats. Diet also influences intestinal flora. *M. major* and *C. rubiginosa* are insectivorous, while *C. perspicillata* are frugivorous. Bacterial content was not significantly influenced by the presence of the pathogenic fungi *Histoplasma capsulatum* (Klite 1965).

10.8.1 Gastrointestinal bacteria in bats of Southeast Asia and Oceania

Banskar *et al*. (2016) examined the intestinal microbial diversity of insectivorous (*Megaderma lyra* and *Rhinolophus* species) and frugivorous (*Cynopterus* species, *Rousettus leschenaultia*, and *Pteropus giganteus*) bats from India using a 16S rRNA

gene library. Of the 47 bacterial species found in frugivorous bats and the 61 species in insectivorous bats, 63 and 59%, respectively, were shared (Banskar *et al*. 2016). A greater extent of bacteria diversity was found in hematophagous and insectivorous bats than in frugivorous or herbivorous bats. The core microbiome revealed five bacterial genera (*Deinococcus, Methylobacterium, Sphingomonas, Phenylobacterium*, and *Hymenobacter*) and members of the *Caulobacteraceae, Streptococcaceae*, and *Chitinophagaceae* families to be present in at least 70% of samples from both types of bats. This may be partially due to dietary overlap since frugivorous bats are known to feed on insects in times of shortages of fruit in order to satisfy their nitrogen requirements (Herrera *et al*. 2001, 2002). This is in agreement with the discovery of members of the *Chitinophagaceae* bacterial family in frugivorous as well as insectivorous bats. These bacteria contain chitin-degrading compounds that help to digest insect exoskeletons that may be consumed by frugivorous bats as a source of nitrogen.

Digestive tract bacteria of short-nosed fruit bats (*Cynopterus brachyotis brachyotis*) from Peninsular Malaysia were enumerated and identified (Daniel *et al*. 2013). These widespread and common bats live in close association with humans in urban settings. The stomachs contained an estimated number of 3×10^{10}–1.4×10^{15} colony forming units (CFU) per milliliter, while intestinal fluid contained $2x10^{10}$–6.1×10^{15} CFU/ml. Sixteen bacterial species from eight genera were identified from this bat species: *Bacillus cereus, Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter hermannii, Enterobacter hormaechei, Enterobacter cloacae, Enterococcus faecalis, Pantoea agglomerans, Pseudomonas aeruginosa, Klebsiella oxytoca, Bacillus thuringiensis, Enterobacter aerogenes, E. coli, Escherichia hermannii, Serratia marcescens*, and *Klebsiella pneumonia*. Most of the isolates were from *Enterobacteriaceae* (12 of the species) and can be pathogenic to humans or wildlife.

E. coli, Citrobacter, Enterobacter, Klebsiella, Pseudomonas, and *Serratia* species were found in the rectum of *Cynopterus sphinx angulatus, Cynopterus brachyotis javanicus*, and *Cynopterus tittaecheilus tittaecheilus* bats in Indonesia (Graves *et al*. 1988). The following bacteria were detected in the rectums of each of four species of flying foxes: alpha-hemolytic and non-hemolytic *Streptococcus, Enterococcus, Corynebacterium*, and *Staphylococcus* species; *E. coli*; and *S. aureus* (Heard *et al*. 1997).

Rats, bats, and feral pigs are the only resident mammals present on any of the Krakatau Islands, having recolonized the islands after the volcanic eruption of 1883. Graves *et al*. (1988) studied fecal bacteria from *Myotis muricola muricola* and *Cynopterus* species. The principle bacterial species in the bats, as well as the rats and pigs, were *Enterobacter, Klebsiella*, and *Citrobacter* species and *E. coli*. Some of the *M. m. muricola* in the Krakatau Islands carried *Morganella* or *Proteus/Providencia* species. *Psuedomonas* and *Serratia* were present in some of the *Cynopterus* bats as well. Interestingly, none of the Krakatau bat stools contained *Staphylococcus faecalis* even though these bacteria were found in bats on nearby, inhabited, West Java.

10.8.2 Gastrointestinal bacteria in bats of Madagascar

Several studies reported finding *Salmonella* species in bats, including some that are linked to human or livestock diseases (Moreno *et al*. 1975; Arata *et al*. 1968). *Salmonella enterica* serotype Typhi was repeatedly isolated from the heart blood, internal organs, and bile of Madagascar flying foxes (*Pteropus rufus*) from a colony in Madagascar

(Brygoo 1973). This serotype is responsible for typhoid fever in humans, a potentially fatal illness characterized by high fever and gastrointestinal symptoms. A more recent cross-sectional study of rectal swabs of 302 *P. giganteus* bats from Bangladesh, however, detected *Salmonella* Virchow from one juvenile female but not *Salmonella* Typhi in a region where human typhoid is common (Islam *et al*. 2013). *Salmonella* Virchow may also cause high fever and gastroenteritis in humans and, occasionally, severe invasive infection or abscesses (Bonalli *et al*. 2011).

Twenty bacterial species were isolated from stools of Malagasy insect eater bats (*Chaerephon pumila*; $n=88$). Most of the species were from the Enterobacteriaceae family. The bacterial species included atypical *Salmonella enterica* subspecies Enterica, *Salmonella enteritidis, Koserella trabulsii, Kluyvera* species, ODC-negative *S. marcescens*, and atypical *Hafnia alvei* (Cassel-Béraud & Richard 1988).

10.8.3 Gastrointestinal bacteria in bats of the Americas

In a study of bacterial diversity in the gastrointestinal tracts of twelve species comprising 377 bats from Trinidad and Tobago, *Campylobacter* species were not detected, but 13.0% of the tested bats were positive for *E. coli*; however, no samples contained the *E. coli* O157 strain, a serious pathogen of humans. Prevalence of antibiotic resistance was high among the *E. coli* isolates (82%). *Salmonella* species were found in 1.1% of the bats as well. The *Salmonella* serotypes included Rubislaw and Molade, both from the fish-eating bat (*Noctilio leporinus*). The Caracas serotype was present in the insectivorous bats *M. major* and *Salmonella* Group I from *Molossus ater* (Adesiyun *et al*. 2009).

A 1964–1966 study of Columbian bats isolated *Salmonella* serotypes from 0.24% of tested bat fecal swabs (Arata *et al*. 1968). *Salmonella* serotype Blockley and Salmonella serotype Anatum were found in insectivorous *Molossus molossus* inhabiting an urban area. *S*. serotype Sandiego was isolated from frugivorous *Artibeus lituratus* in the rain forest of the Columbian Pacific Coast and the rare *Salmonella* serotype Llandoff from frugivourous *Sturnira lilium* from a fruit plantation. A separate 1965 study found *Salmonella* serotype Typhimurium and *Salmonella* serotype Saintpaul in *G. soricina* bats in Panama (Klite & Kourany 1965). *S*. serotype Typhimurium is a human pathogen which causes gastroenteritis and diarrhea. The Columbian study also isolated *Shigella boydii-2* from insectivorous *Molossus bondae* in a region at high altitude. This is noteworthy because *Shigella* is rarely isolated in nonhuman mammals (Arata *et al*. 1968).

A study conducted in Brazil of stools of the hematophageous common vampire bat (*Desmodus rotundus*; $n=100$) found 29.5% contained hemolytic and non-hemolytic strains of *E. coli*, 27% contained *Proteus* species, 20% had *Staphylococcus* species, and 9% had *S*. serotype Typhimurium (Moreno *et al*. 1975). A survey of Gram-negative aerobic bacteria of German bats yielded different findings (Pinus & Müller 1980). It is not surprising to find that the intestinal contents of hematophageous species differ greatly from frugivorous or insectivorous bats. All *D. rotundus* fecal flora contained *Aeromonas hydrophila*, either as a pure culture or in conjunction with *E. coli, Enterobacter, Providencia*, or *Salmonella* sertotype Arizona. The high incidence of *A. hydrophila* in *D. rotundus* feces suggests that these bacteria may aid in digestion of drunken blood contents. This study also found 15–24% of insectivorous and frugivorous bats' feces contained *E. coli*, 8–10% had *Citrobacter*, 40–43% had members of the *Enterobacter–Klebsiella*

group, and 28–30% contained *Proteus* species, with no specific differences seen between the insectivorous and frugivorous animals (Pinus & Müller 1980).

Twenty-six bacterial species were found in oral and anal samples from 502 frugivorous, 29 hematophagous, and 11 nectivorous bats from Mexico. The predominant phylum was Proteobacteria and members of the Enterobacteriaceae family. Significant differences were seen between oral and anal samples (Galicia *et al*. 2014). Some bacterial specificity was seen: *B. cereus* in nectivorous and frugivorous bats and *P. aeruginosa, S. marcescens, S. aureus, Staphylococcus epidermis*, and *A. hydrophyla* in hematophagous bats. At least part of the microbiota may be mutualistic, providing bats with supplemental nutrients, contributing to immune system development, stabilizing microbial populations, and avoiding colonization by pathogens.

10.9 LARGE-SCALE STUDIES OF OTHER BAT BACTERIA

A study of approximately 500 deceased free-range bats from 19 European species (family Vespertilionidae) in Germany found inflammatory lesions in more than half of the animals, with 40% of the lesions present in the lungs, irrespective of bat species, sex, and age (Mühldorfer *et al*. 2009). Twenty-two bacterial species were associated with the pathological lesions. Lung inflammatory lesions were mild to severe, with interstitial pneumonia in almost 38% of these bats and consisted of mixed neutrophilic and mononuclear infiltration of alveolar septa. Approximately 23% of the interstitial pneumonia was seen in conjunction with infection by members of the bacterial families *Pasteurellaceae, Enterobacteriaceae*, and *Streptococcaceae*. Pulmonary lesions due to secondary infection with *B. cillus cereus* were present in a small number of bats. Fatal thoracic cavity effusions were associated with *P. multocida* infection of the heart and thoracic cavity in several parti-colored bats (*V. murinus*). Liver lesions, including necrosis, were found in 11% of the animals in the presence of *P. multocida, Pasteurella* species B, and *Y. pseudotuberculosis*. Splenic lesions were present in 2% of the bats in the presence of *P. multocida* or *Y. pseudotuberculosis* and urinary system lesions of moderate to severe suppurative necrotizing nephritis were associated with systemic *P. multocida* or *E. coli* infection. Other vespertilionid bats (*P. pipistrellus, P. nathusii, N. noctula, M. mystacinus, Myotis brandtii*, and *E. serotinus*) also had renal coccidiosis with mild to severe cystic tubular dilatation. One animal had mild purulent meningitis and encephalitis in the presence of generalized *S.* serotype *Typhimurium* infection.

From samples of 19 species of 430 deceased German *Vespertilionidae* bats, 42 bacterial genera were identified. The most prevalent bacterial species were *E. faecalis* from 14.7% of the bats, *H. alvei* from 11.2% of the bats, *Serratia liquefaciens* from 10% of the bats, and *P. multocida* from 7.7% of the bats (Mühldorfer *et al*. 2011a). Infection with 22 of the identified bacterial species was associated with pathological lesions or systemic infection in 17% of the bats. *Pasteurellaceae, Enterobacteriaceae*, and *Streptococcaceae* were most often associated with pathology in bats. Many of these bacterial species are opportunistic pathogens and are more likely to cause disease in already-traumatized animals. However, primary pathogens like *Samonella enterica* serovar Typhimurium, *S. enteritidis*, and *Y. pseudotuberculosis* were present in approximately 12% of the bats. Members of Vespertilionidae have also been found to harbor the following bacteria: *Campylobacter jejuni, Clostridium perfringens, Clostridium sordellii, Listeria* species, *S. typhimurium, Shigella flexneri, Vibrio* species, and *Y. enterocolitica* (reviewed by

Mühldorfer *et al*. 2011b). Some of these bacteria are pathogenic to bats and may cause severe dysentery in humans. *C. sordellii* and *C. perfringens* are the primary causes of hemorrhagic diarrhea in European vespertilionid bats (Mühldorfer *et al*. 2011a). Of note, *C. sordellii* was present in a moribund *M. mystacinus* found in close proximity to humans and in a group of ill, captive *Nyctalus noctule*.

Bat urine or organs of the family Molossidae were also infected with the following: *Clostridium* species*, Listeria monocytogenes, Salmonella* Caracas, *Salmonella* Anatum, *Salmonella* Blockley, *Salmonella* Enteritidis, *Salmonella* O48, and *S. boydii-2* (reviewed by Mühldorfer *et al*. 2011b). Phyllostomidae bats were found to be infected by the following: *S*. Typhimurium, *Salmonella sonnei, Clostridium* species, *Salmonella* Saintpaul, *Salmonella* Typhimurium var. Copenhagen, *Salmonella* Llandoff, and *Salmoella* Sandiego. Pteropodidae bats were found to be infected by the following*: L. monocytogenes, Salmonella* Typhi, *S*. Typhimurium*, S*. Enteritidis*, Salmonella flexneri*, and *S. sonnei*, while members of the bat family Noctilionidae contained *Salmonella* Molade, and *Salmonella* Rubislaw (reviewed by Mühldorfer *et al*. 2011b). *S*. Enteritidis and *S*. Typhimurium have been found in dead or severely injured bats of the Vespertilionidae family. These bats had inflammatory lesions in multiple organs as well as interstitial pneumonia and purulent meningitis. *S. sonnei* is found in both mega- and microbats with diverse feeding habitats. *S. flexneri* and *S. sonnei* are responsible for most of the human shigellosis cases worldwide (reviewed by Mühldorfer 2013). In addition to causing potentially severe or fatal disease in humans, many of these bacteria also cause intestinal or extra-intestinal bacterial disease, including kidney damage.

10.10 BACTERIAL SPECIES BENEFICIAL TO BATS

Some bacterial species isolated from bat skin may prove to be very beneficial in the fight against white-nose syndrome. When six isolates of *Pseudomonas* from the skin of *E. fuscus* and *M. lucifugus* were co-cultured with *Psuedogymnoascus destructans*, zones of inhibition were seen and fungal growth was significantly inhibited for at least 35 days (Hoyt *et al*. 2015). These bacteria were most closely related to the mobile *Pseudomonas fluorescens*.

The major food source of the Indian flying fox (*P. giganteus*) consists of fruit and leaves which are composed, to a large degree, of the glucose-containing polysaccharides cellulose and xylan. In order to derive carbohydrates from the leaves, these bats need to harbor cellulolytic and xylanolytic bacteria in their digestive tracts. Several such bacterial species were isolated from *P. giganteus*. These bacteria are the Gram-negative bacilli *Proteus vulgaris, Proteus mirabilis, Citrobacter freundii, S. liquefaciens, K. oxytoca* and *Spironema* (*Treponema*) species. *Treponema saccharophilum* has sacchrolytic activity in ruminates' guts and other members of the *Treponema* may perform this function in bats (Kasperowicz & Michalowski 2002; Anand & Sripathi 2004). These bacterial species have not been found in the insectivorous bat *Hipposideros fulvus*. *Enterobacter* species, common in the intestines of some types of frugivorous bats, break down most sugars, including xylose (Daniel *et al*. 2013). Sphingobacteriaceae are also present in bat guts and degrade various organic polymers (Thomas *et al*. 2011). Enterobacteriaceae are other common inhabitants of animal intestines. Some unclassified members of Enterobacteriaceae from frugivorous bats ferment sugar.

In insectivorous bats, members of the bacterial Clostridiaceae family aid in carbohydrate fermentation (Wiegel *et al*. 2006). Specifically, *Clostridium beijerinckii* are able to

at least partially digest *N*-acetylglucosamine, a component of chitin (Makishah & Mitchell 2013), in addition to Chitinophagaceae bacteria, as mentioned above. Bacterial interaction analysis has also shown that *Phenylobacterium, Hymenobacter, Methylobacterium, Deinococcus, Sphingomonas*, and *Chitinophaga* are positively associated and may assist the growth or survival of each other (Banskar *et al*. 2016). Chitin-degrading bacteria may make chitin by-products available to the other associated bacteria (Raes & Bork 2008).

10.11 CONCLUSIONS

Study of the microbiome of humans and animals is a relatively new and exciting field of inquiry. Many gut bacteria, including many strains of *E. coli*, play an important role in producing the host organism with vitamins, assisting in digestion, and regulating metabolism and immune functioning. Some bacteria may help to protect bats from white-nose syndrome by killing *G. destructans*. Other bacteria are cellulolytic or xylanolytic and break down complex plant carbohydrates, including cellulose, while other bacteria contain enzymes that degrade chitin, found in insect exoskeletons.

Many pathogenic species or subspecies of bacteria, however, cause mild to life-threatening infections. A development of particular concern is the increasing trend towards antibiotic resistance. An example of this situation is seen in *E. coli*, in which 46% of tested Mexican bats showed resistance to ampicillin and 100%, to streptomycin. If a highly pathogenic bacterial strain were to become antibiotic-resistant, the resulting infection could be very serious. The fact that some bacteria undergo interspecies transmission exacerbates the problem. Zoonotic infection of *E. coli* from bats to humans, however, is likely to occur seldom, if ever, since the major means of transmission to humans is ingestion of water or food contaminated by cattle or human feces containing the pathogenic O157:H7 strain. Of greater concern, MRSA has been detected in a European bat.

Leptospira species bacteria are currently the most common causative agents of zoonotic infection in the world. Humans are infected by *Leptospira interrogans*, which uses murid rats as its vector. Members of the bat family Phyllostomidae in the Peruvian Amazon, however, also carry pathogenic *Leptospira* species, particularly adult bats residing in mature forest environments rather than in urban centers. In addition to bats, a high prevalence of infection is seen in urban dogs and rats, suggesting that these animals are more likely responsible for zoonotic infection of humans. Interestingly, despite the lack of human leptospirosis in Madagascar and Union of the Comoros, *Leptospira* DNA was found in eleven of twelve Madagascar bat species, demonstrating a lack of correlation between infection in bats and humans in this rather unique region of the world. *Rattus rattus* is the major reservoir of leptospiridians in Madagascar. In Australia, leptospirial DNA was found in the kidneys or urine of all four species of flying foxes. Taken together, the above information suggests that despite the high prevalence of *Leptospira* infection in bats in some areas of the world, urban rats may be the primary source of zoonotic infection in humans.

Several species of *Yersinia* are highly pathogenic to humans and bats, particularly *Y. pseudotuberculosis* and *Y. enterocolitica*. Infection of *M. myotis* or *R. aegyptiacus* by *Y. pseudotuberculosis* causes severe disease, including abscesses in the liver, spleen, kidneys, and lungs and death. Stress appears to increase disease severity in bats. Birds and rodents are believed to be the primary reservoirs of *Y. pseudotuberculosis*.

A large number of *Pasteurella* species infect bats and cause subcutaneous abscesses and, in some cases, pneumonia and death. Cats and dogs carry *Pasteurella* as part of

their normal nasopharyngeal flora without pathological consequences. Many infected bats show signs of cat predation, implicating cat bites as a major route of *Pasteurella* transmission to bats. Humans also may develop skin abscesses following the bite of an infected cat.

Mycoplasma are atypical bacteria which lack cell walls and are extremely small. They serve as a causative agent of infectious hemolytic anemia that may be life-threatening, particularly if the host is co-infected by another pathogenic microbe, such as *G. destructans*. Several species of bats are known to be infected by one of two mycoplasma species, including the North American *Myotis lucifugus, M. capaccinii*, and *Miniopterus schreibersii*. Some of these unusual bacteria belong to a phylogenetic branch that contains mycoplasma of humans as well as bats, thus interspecies transmission of these bacteria to humans may be possible.

Two species of *Waddlia* have been isolated from a nectivorous bat species from Malaysia and a frugivorous bat species from tropical regions of the Americas. *Waddlia* species are similar to chlamydia and are obligate intracellular bacteria. Prior to its death, the New World bat was emaciated, restless, depressed, and had pallor on its wings. Some experimentally infected bats have developed severe multifocal interstitial pneumonia and diffuse lymphoid hyperplasia in the spleen. *Waddlia* species found in humans cause miscarriages.

Large studies of gut bacteria in bats found that the degree of bacterial diversity positively correlated with overall bat health. As expected, diet influences the gut bacteriome. Hematophagous and insectivorous bats have a greater degree of bacterial diversity than frugivorous or herbivorous bats. Overlap in the bacteriomes of these groups of bats may be partially due to the consumption of insects during feeding of frugivorous bats. Some of the bacteria in bat guts are pathogenic to humans, including *Salmonella* serotypes Typhi, Typhimurium, and Virchow, and a variety of *Enterobacteriaceae*. Many bacteria found in a variety of internal organs caused mild to severe pathology in their bat hosts, including many of the above human pathogens. Bacterial infection of bats has been linked to severe interstitial pneumonia (due to infection by *Pasteurella, Enterobacter*, and *Streptococcus* species), liver and splenic lesions (*Pasteurella* and *Yersinia* species), severe suppurative necrotizing nephritis and severe cystic tubular dilatation (systemic infection by *P. multocida* or *E. coli*; renal coccidia), purulent meningitis or encephalitis (*Salmonella* serotype Typhimurium), and hemorrhagic diarrhea (*Clostridium* species).

Bats play host to numerous species of bacteria, as is the case for other groups of vertebrates. Some of these are beneficial to their hosts, while others are associated with severe pathology or death in bats. Some of the bacteria found in bat urine or feces are also highly pathogenic to humans. While bats may serve as reservoir hosts or vectors for some human pathogens, rodents are implicated as having a much greater role in zoonotic transmission for many of the above bacterial species.

REFERENCES

Adesiyun AA, Stewart-Johnson A, Thompson NN. 2009. Isolation of enteric pathogens from bats in Trinidad. *Journal of Wildlife Diseases.* 45(4):952–961.

Anand AA, Sripathi K. 2004. Digestion of cellulose and xylan by symbiotic bacteria in the intestine of the Indian flying fox (*Pteropus giganteus*). *Comparative Biochemistry and Physiology.* 139:65–69.

Arata AA, Vaughn JB, Newell KW, Barth RAJ, Gracian M. 1968. *Salmonella* and *Shigella* infections in bats in selected areas of Columbia. *American Journal of Tropical Medicine and Hygiene.* 17:92–95.

Banskar S, Mourya DT, Shouche YS. 2016. Bacterial diversity indicates dietary overlap among bats of different feeding habits. *Microbiological Research.* 182:99–108.

Beltz LA. 2011. *Foundations of Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey-Bass and APHA Press: San Francisco, CA.

Bessa TAF, Spichler A, Chapola EGB, Husch AF, de Almeida MF, Sodré MM, Savani ESMM, Sacramento DRV, Vinetz JM. 2010. The contribution of bats to leptospirosis transmission in São Paulo City, Brazil. *American Journal of Tropical Medicine and Hygiene.* 82(2):315–317.

Bonalli M, Stephan R, Käppeli U, Cernela N, Adank L, Hächler H. 2011. *Salmonella enterica* serotype Virchow associated with human infections in Switzerland: 2004–2009. *BMC Infectious Diseases.* 11:49.

Brygoo ER. 1973. Les chiropteres réservoirs de virus d'entérobactéries pathogenes pour l'homme. *Archives de I'Institut Pasteur de Madagascar.* 42:239–252.

Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM. 2000. Detection of pathogenic *Leptospira* spp. infections among mammals captured in the Peruvian Amazon Basin Region. *American Journal of Tropical Medicine and Hygiene*. 63(5,6):255–258.

Cassel-Béraud AM, Richard C. 1988. The aerobic intestinal flora of the microchiropteran bat *Chaerephon pumila* in Madagascar. *Bulletin of the Society for Pathology for Exotic Filiates.* 81(5):806–810.

Childs-Sanford SE, Kollias GV, Abou-Madi N, McDonough PL, Garner MM, Mohammed HO. 2009. *Yersinia pseudotuberculosis* in a closed colony of Egyptian fruit bats (*Rousettus aegyptiacus*). *Journal of Zoo and Wildlife Medicine.* 40(1):8–14.

Chua PKB, Corkill JE, Hooi PS, Cheng SC, Winstanley C, Hart C. 2005. Isolation of *Waddlia malaysiensis*, a novel intracellular bacterium, from fruit bat (*Eonycteris spelaea*). *Emerging Infectious Diseases.* 11(2):271–277.

Collins FM. 1996. *Pasteurella, Yersinia,* and *Francisella*. In: *Medical Microbiology*, 4th edition. S Baron (ed.). University of Texas Medical Branch at Galveston: Galveston, TX, Chapter 29.

Cox TE, Smythe LD, Leung LK-P. 2005. Flying foxes as carriers of pathogenic *Leptospira* species. *Journal of Wildlife Diseases.* 41(4):753–757.

Cummings JH, Macfarlane GT. 1997. Role of intestinal bacteria in nutrient metabolism. *Journal of Parenteral and Enteral Nutrition.* 21(6):357–365.

Daniel DS, Ng YK, Chua EL, Arumugam Y, Wong YL, Kumaran JV. 2013. Isolation and identification of gastrointestinal microbiota from the short-nosed fruit bat *Cynopterus brachyotis brachyotis*. *Microbiological Research.* 168:485–496.

Dietrich M, Wilkinson DA, Benlali A, Lagadec E, Ramasindrazana B, Dellagi K, Tortosa P. 2015. *Leptospira* and paramyxovirus infection dynamics in a bat maternity enlightens pathogen maintenance in wildlife. *Environmental Microbiology.* 17(11):4280–4289.

Dietrich M, Wilkinson DA, Soarimalala V, Goodman SM, Dellagi K, Tortosa P. 2014. Diversification of an emerging pathogen in a biodiversity hotspot: *Leptospira* in endemic small mammals of Madagascar. *Molecular Ecology.* 23:2783–2796.

Galicia MM, Buenrostro A, García J. 2014. Specific bacterial diversity in bats of different food guilds in Southern Sierra Oaxaca, Mexico. *Reviews of Biological Tropics.* 62(4):1673–1681.

Graves SR, Kennely-Merrit SA, Tidemann CR, Rawlinson PA, Harvey KJ, Thornton IWB. 1988. Antibiotic-resistance patterns of enteric bacteria of wild mammals on the Krakatau Islands and West Java, Indonesia. *Philosophical Transactions of the Royal Society of London B*. 322:339–353.

Harkin KR, Hays M, Davis R, Moore M. 2014. Use of PCR to identify *Leptospira* in kidneys of big brown bats (*Eptesicus fuscus*) in Kansas and Nebraska, USA. *Journal of Wildlife Diseases.* 50(3):651–654.

Heard DJ, De Young JL, Goodyear B, Ellis GA. 1997. Comparative rectal bacterial flora of four species of flying fox (*Pteropus* sp.). *Journal of Zoo and Wildlife Medicine*. 28:471–475.

Helmick KE, Heard DJ, Richey L, Finnegan M, Ellis GA, Nguyen A, Tucker L, Weyant RS. 2004. A *Pasteurella*-like bacterium associated with pneumonia in captive megachiropterans. *Journal of Zoo and Wildlife Medicine.* 35(1):88–93.

Herrera LG, Gutierrez E, Hobson KA, Altube B, Díaz WG, Sánchez-Cordero V. 2002. Sources of assimilated protein in five species of New World frugivorous bats. *Oecologia.* 133:280–287.

Herrera LG, Hobson KA, Mirón ML, Ramírez NP, Méndez GC, Sánchez-Cordero V. 2001. Sources of protein in two species of phytophagous bats in a seasonal dry forest: evidence from stable-isotope analysis. *Journal of Mammalogy.* 82:352–361.

Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF, Kilpatrick AM. 2015. Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS ONE.* 10(4):e0121329.

Islam A, Mikolon A, Mikoleit M, Ahmed D, Khan SU, Sharker MAY, Hossain MJ, Islam A, Epstein JH, Zeidner N, Luby SP. 2013. Isolation of *Salmonella* Virchow from a fruit bat (*Pteropus giganteus*). *EcoHealth.* 10:348–351.

Kasperowicz A, Michalowski T. 2002. Assessment of the fructanolytic activities in the rumen bacterium *Treponema saccharophilum* strain S. *Journal of Applied Microbiology.* 92:140–146.

Klite PD. 1965. Intestinal bacterial flora and transit time of three neotropical bat species. *Journal of Bacteriology.* 90(2):375–379.

Klite PD, Kourany M. 1965. Isolation of salmonellae from a neotropical bat. *Journal of Bacteriology.* 90:831.

Lagadec E, Gomard Y, Guernier V, Dietrich M, Pascalis H, Temmam S, Ramasindrazana B, Goodman SM, Tortosa P, Dellagi K. 2012. Pathogenic leptospira spp. in bats, Madagascar and Union of the Comoros. *Emerging Infectious Diseases*. 18(10):1696–1698.

Lei BR, Olival KJ. 2014. Contrasting patterns in mammal–bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. *PLoS Neglected Tropical Diseases.* 8(3):e2738.

Makishah NH, Mitchell WJ. 2013. Dual substrate specificity of an N-acetylglucosamine phosphotransferase system in *Clostridium beijerinckii*. *Applied Environmental Microbiology.* 79:6712–6718.

Mascarelli PE, Keel MK, Yabsley M, Last LA, Breitschwerdt EB, Maggi RG. 2014. Hemotropic mycoplasmas in little brown bats (*Myotis lucifugus*). *Parasites & Vectors.* 7:117.

Matthias MA, Díaz MM, Campos KJ, Calderon M, Willig MR, Pacheco V, Gotuzzo E, Gilman RH, Vinetz JM. 2005. Diversity of bat *Leptospira* in the Peruvian Amazon inferred by Bayesian phylogenetic analysis of 16 s ribosomal DNA sequences. *American Journal of Tropical Medicine and Hygiene.* 73(5):964–974.

Mgode GF, Mbugi HA, Mhamphi GG, Ndanga D, Nkwama EL. 2014. Seroprevalence of Leptospira infection in bats roosting in human settlements in Morogoro municipality in Tanzania. *Tanzanian Journal of Health Research.* 16(1):23–28.

Moreno G, Lopes CA, Seabra E, Pavan C, Corrêa A. 1975. Bacteriological study of the intestinal flora of bats (*Desmodus rotundus*). *Arquivos do Instituto Biologico (São Paulo)*. 42:229–232.

Millán J, López-Roig M, Delicado V, Serra-Cobo J, Esperón F. 2015. Widespread infection with hemotropic mycoplasmas in bats in Spain, including a hemoplasma closely related to "*Candidatus* Mycoplasma hemohominis." *Comparative Immunology, Microbiology and Infectious Diseases.* 39:9–12.

Mühldorfer K. 2013. Bats and bacterial pathogens: a review. *Zoonoses and Public Health.* 60:93–103.

Mühldorfer K, Schwarz S, Fickel J, Wibbelt G, Speck S. 2011a. Genetic diversity of *Pasteurella* species isolated from European vespertilionid bats. *Veterinary Microbiology*.149:163–171.

Mühldorfer K, Speck S, Kurth A, Lesnik R, Freuling C, Müller T, Kramer-Schadt S, Wibbelt G. 2011b. Diseases and causes of death in European bats: dynamics in disease susceptibility and infection rates. *PLoS ONE.* 6(12):e29773.

Mühldorfer K, Speck S, Wibbelt G. 2009. Diseases in free-ranging bats from Germany. *BMC Veterinary Research.* 7:61.

Mühldorfer K, Speck S, Wibbelt G. 2014. Proposal of *Vespertiliibacter pulmonis* gen. nov., sp. nov. and two genomospecies as new members of the family *Pasteurellaceae* isolated from European bats. *International Journal of Systematic and Evolutionary Microbiology*. 64:2424–2430.

Mühldorfer K, Wibbelt G, Haensel J, Riehm S, Speck S. 2010. *Yersinia* species isolated from bats, Germany. *Emerging Infectious Diseases.* 16:578–580.

Ogawa H, Koizumi N, Ohnuma A, Mutemwa A, Hang'ombe BM, Mweene AS, Takada A, Sugimoto C, Suzuki Y, Kida H, Sawa H. 2015. Molecular epidemiology of pathogenic *Leptospira* spp. in the straw-colored fruit bat (*Eidolon helvum*) migrating to Zambia from the Democratic Republic of Congo. *Infection, Genetics and Evolution*. 32:143–147.

Pierlé SA, Morales CO, Martínez LP, Ceballos NA, Rivero JJP, Díaz OL, Brayton KA, Setién AA. 2015. Novel *Waddlia* intracellular bacterium in *Artibeus intermedius* fruit bats, Mexico. *Emerging Infectious Diseases.* 21(12):2161–2163.

Pinus M, Müller HE. 1980. Enterobacteria of bats (*Chiroptera*). *Zentralblatt für Bakteriologie A.* 247(3):315–322.

Raes J, Bork P. 2008. Molecular eco-systems biology: towards an understanding of community function. *Nature Reviews of Microbiology.* 6:693–699.

Scott KP, Antoine JM, Midtvedt T, and van Hemert S. 2015. Manipulating the gut microbiota to maintain health and treat disease. *Microbiology and Ecology in Health and Disease*. 26:25877.

Simpson VR. 2000. Veterinary advances in the investigation of wildlife diseases in Britain. *Research in Veterinary Science.* 69:11–16.

Smythe LD, Field HE, Barnett LJ, Smith CS, Dohnt MF, Symonds ML, Moore MR, Rolfe PF. 2002. Leptospiral antibodies in flying foxes in Australia. *Journal of Wildlife Diseases.* 38(1):182–186.

Steer JA, Tasker S, Barker EN, Jensen J, Mitchell J, Stocki T, Chalker VJ, Hamon M. 2011. A novel hemotropic Mycoplasma [hemoplasma] in a patient with hemolytic anemia and pyrexia. *Clinical Infectious Diseases.* 53:e147–151.

Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G. 2011. Environmental and gut Bacteroidetes: the food connection. *Frontiers in Microbiology.* 2(93):1–16.

Tulsiani SM, Cobbold RN, Graham GC, Dohnt MF, Burns MA, Leung LK, Field HE, Smythe LD, Craig SB. 2011. The role of fruit bats in the transmission of pathogenic leptospires in Australia. *Annals of Tropical Medicine and Parasitology.* 105(1):71–84.

Vashi NA, Reddy P, Wayne DB, Sabin B. 2009. Bat-associated leptospirosis. *Journal of General Internal Medicine.* 25(2):162–164.

Wiegel J, Tanner R, Rainey F. 2006. An introduction to the family Clostridiaceae. In: *The Prokaryotes SE-20*. M Dworkin, S Falkow, E Rosenberg, K-H Schleifer and E Stackebrandt (eds). Springer: New York, NY, pp. 654–678.

IV

PROTIST INFECTIONS OF BATS

11

APICOMPLEXANS AND BATS

11.1 INTRODUCTION TO APICOMPLEXA AND COCCIDEA

Apicomplexa is one of the phyla of protozoans, single-celled forms of life. All members of the phylum are parasitic. These organisms are characterized by the presence of an apical complex at some stage of their life cycle. Asexual reproduction alternates with sexual reproduction. The latter form of reproduction utilizes syngamy, fusion of the larger macrogamete with the smaller microgamete. Asexual reproduction involves multiple fission (schizogony) to form a multinucleated cell known as the shizont or meront. During the process of merogony, the shizont's daughter cells are arranged around the schizont's periphery. A membrane forms around each nucleus, followed by cytoplasmic division. The newly formed separate organisms, the merozoites, are composed of the nucleus and its attendant cytoplasm surrounded by the membrane. The merozoites then break out of the ruptured cell and infect new host cells. After entry into the host cells, the merozoites may either undergo another round of schizongy or transform into a macro- or microgametocyte via gametogamy to produce the macro- or microgamont.

Apicomplexa is divided into two classes: Gregarinea and Coccidea. Gregarinea do not infect vertebrates, while many Coccidea use vertebrate intermediate hosts during their life cycles, infecting their digestive tract epithelium, liver, kidneys, or blood cells. Bat parasites have been reported in four of the coccidian orders: Haemosporida, Piroplasmida, Eimeriida, and Adeleida (Roberts & Janovy Jr 2006). See Table 11.1 for a list of various apicomplexans with reported association to bats.

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 11.1 Apicomplexans associated with bats

Bat family	Bat common name	Bat species	Apicomplexan (Reference)
Vespertilionidae	Pallid bat	*Antrozous pallidus*	*Eimeria antrozoi* (Zhao *et al*. 2001)
Phyllostomidae	Fringed fruit-eating bat	*Artibeus fimbriatus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Toxoplasma gondii* (Fournier *et al*. 2014)
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Rhinolophidae	Stoliczka's trident bat	*Aselliscus stoliczkanus*	*Cryptosporidium* genotype I (Wang *et al*. 2013)
Rhinolophidae	Stoliczka's trident bat	*Aselliscus stoliczkanus*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Phyllostomidae	Seba's short-tailed fruit bat	*Carollia perspicillata*	*Toxoplasma gondii* (Fournier *et al*. 2014)
Vespertilionidae	Gland-tailed free-tailed bat	*Chaerephon bemmeleni*	*Eimeria levinei* (Bray 1958)
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	*Hepatocystis pteropid* (Masbar *et al*. 1981)
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	*Cryptosporidium* genotype VI (Murakoshi *et al*. 2016)
Pteropodidae	Lesser short-nosed fruit bat	*Cynopterus brachyotis*	*Eimeria* sp. (Murakoshi *et al*. 2016)
Pteropodidae	Horsfield's short-nosed bat	*Cynopterus horsfieldi*	*Hepatocystis pteropid* (Masbar *et al*. 1981)
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx*	*Hepatocystis garnhami* (Landau 1981)
Pteropodidae	Greater short-nosed fruit bat	*Cynopterus sphinx*	*Toxoplasma gondii* (Dodd *et al*. 2014)
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Toxoplasma gondii* (Cabral *et al*. 2013)
Pteropodidae	Muluccan naked-backed fruit bat	*Dobsonia moluccensis*	*Hepatocystis pteropi* manwelli (Garnham 1966)
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	*Eimeria* sp. (Murakoshi *et al*. 2016)
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Pteropodidae	Cave nectar bat	*Eonycteris spelaea*	*Cryptosporidium* genotype VII (Murakoshi *et al*. 2016)
Molossidae	Buettikofer's epaulettted fruit bat	*Epomops buettikoferi*	*Hepatocystis* sp. (Schaer *et al*. 2013)
Molossidae	Franquet's epauletted fruit bat	*Epomops franqueti*	*Hepatocystis brosset* (Miltgen *et al*. 1977)
Molossidae	Franquet's epauletted fruit bat	*Epomops franqueti*	*Hepatocystis epomophori* (Rodhain 1926)
Pteropodidae	Gambian epauletted fruit bat	*Epomophorus gambianus*	*Hepatocystis* (Schaer *et al*. 2013)
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Bioccala deanei* (Garnham *et al*. 1971)
Vespertilionidae	Big brown bat	*Estesicus fuscus*	*Nephroisospora eptesicino* (Wünschmann *et al*. 2010)
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Crytoptosporideum parvum* genotype III (Kváč *et al*. 2015)
Molossidae	Black bonneted bat	*Eumops auripendus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Molossidae	Wagner's bonneted bat	*Eumops glaucinus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Molossidae	Western bonneted bat	*Eumops perotis*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Toxoplasma gondii* (Fournier *et al*. 2014)
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	*Hepatocystis* sp. (Manwell & Kuntz 1966)
Hipposideridae	Great roundleaf bat	*Hipposideros armiger*	*Toxoplasma gondii* (Sun *et al*. 2013)

Hipposideridae	Bicolored roundleaf bat	*Hipposideros bicolor*	*Bioccala* sp. (Eyles *et al*. 1962)
Hipposideridae	Sundevall's roundleaf bat	*Hipposideros caffer*	*Klossiella killicki* (Bouland 1975)
Hipposideridae	South East Asian bat	*Hipposideros cervinus*	*Hepatozoon* (Pinto *et al*. 2013)
Hipposideridae	Cyclops roundleaf bat	*Hipposideros cyclops*	*Dionisia bunoi* (Landau 1980a)
Hipposideridae	Cyclops roundleaf bat	*Hipposideros cyclops*	*Plasmodium cyclopsi* (Landau & Chabaud 1978)
Hipposideridae	Fulvus roundleaf bat	*Hipposideros fulvus*	*Cryptosporidium* genotype II (Wang *et al*. 2013)
Hipposideridae	Fulvus roundleaf bat	*Hipposideros fulvus*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Hipposideridae	Cantor's roundleaf bat	*Hipposideros galeritus*	*Hepatocystis bainae* (Mialhe & Landau 1977)
Hipposideridae	Cantor's roundleaf bat	*Hipposideros galeritus*	*Hepatocystis rodhaini* (Landau *et al*. 1976)
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	*Biguetiella minuta* (Landau *et al*. 1984)
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	*Hepatocystis* sp. (Duval *et al*. 2007)
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	*Nycteria brucechwatti* (Landau *et al*. 1984)
Hipposideridae	Intermediate roundleaf bat	*Hipposideros larvatus*	*Toxoplasma gondii* (Qin *et al*. 2014)
Hipposideridae	Noack's roundleaf bat	*Hipposideros ruber*	*Nycteria* sp. (Schaer *et al*. 2015)
Vespertilionidae	Tropical big-eared brown bat	*Histiotus velatus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Pteropodidae	Hammerhead bat	*Hypsignatus monstrosus*	*Hepatocystis carpenter* (Miltgen *et al*. 1980)
Vespertilionidae	Hardwicke's forest bat	*Kerivoula hardwickii*	*Haemosporidia* sp. (Duval *et al*. 2007)
Vespertilionidae	Silver-haired bat	*Lasionycteris noctivagans*	*Eimeria catronensis* (Seville & Gruver 2004)
Vespertilionidae	Eastern red bat	*Lasiurus borealis*	*Eimeria dowleri* (McAllister & Upton 2009)
Vespertilionidae	Eastern red bat	*Lasiurus borealis*	*Eimeria sealanderi* (McAllister & Upton 2009)
Pteropodidae	None	*Lyssonycteris smithi*	*Plasmodium voltaicum* (Van der Kaay 1964)
Megadermatidae	Big-eared bat	*Megaderma lyra*	*Toxoplasma gondii* (Yuan *et al*. 2013)
Megadermatidae	Lesser false vampire bat	*Megaderma spasma*	*Haemosporidia* sp. (Duval *et al*. 2007)
Megadermatidae	Lesser false vampire bat	*Megaderma spasma*	*Nycteria* sp. (Duval *et al*. 2007)
Pteropodidae	Peters's dwarf epauletted fruit bat	*Micropteropus pusillus*	*Hepatocystis* sp. (Schaer *et al*. 2013)
Miniopteridae	Japanese long-fingered bat	*Miniopterus fuliginosus*	*Toxoplasma gondii* (Sun *et al*. 2013)
Miniopteridae	Glen's long-fingered bat	*Miniopterus gleni*	*Haemosporidia* sp. (Duval *et al*. 2007)
Miniopteridae	Greater long-fingered bat	*Miniopterus inflatus*	*Polychromophilus melanipherus* (Duval *et al*. 2012)
Miniopteridae	Greater long-fingered bat	*Miniopterus inflatus*	*Polychromophilus corradettii* (Landau *et al*. 1980b)
Miniopteridae	Manavi long-fingered bat	*Miniopterus manavi*	*Polychromophilus* sp. (Megali *et al*. 2011)
Miniopteridae	Manavi long-fingered bat	*Miniopterus manavi*	*Haemosporidia* sp. (Duval *et al*. 2007)
Miniopteridae	Least long-fingered bat	*Miniopterus minor*	*Polychromophilus adami* (Landau *et al*. 1980b)
Miniopteridae	Least long-fingered bat	*Miniopterus minor*	*Bioccala murinus* (Dionisi 1899)
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	*Bioccala murinus* (Dionisi 1899)
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	*Polychromophilus* corradettii (Dionisi 1899)

(*Continued*)

TABLE 11.1 (Continued)

Bat family	Bat common name	Bat species	Apicomplexan (Reference)
Miniopteridae	Schreiber's long-fingered bat	*Miniopterus schreibersi*	*Polychromophilus melanipherus* (Dionisi 1899)
Miniopteridae	Villiers' long-fingered bat	*Miniopterus villiersi*	*Polychromophilus melanipherus* (Schaer *et al*. 2015)
Molossidae	Velvety free-tailed bat	*Molossus molossus*	*Toxoplasma gondii* (Cabral *et al*. 2013)
Molossidae	Rufous' dog-faced bat	*Molossops neglectus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Mormoopidae	Leaf-chinned bats	*Mormoops megalophylla*	*Babesia vesperuginis* (Marinkelle 1996)
Vespertilionidae	Greater tube-nosed bat	*Murina leucogaster*	*Toxoplasma gondii* (Qin *et al*. 2014)
Pteropodidae	Angolan rousette	*Myonycteris angolensis*	*Plasmodium voltaicum* (Schaer *et al*. 2013)
Pteropodidae	Sierra Leone collared fruit bat	*Myonycteris leptodon*	*Hepatocystis* species (Schaer *et al*. 2013)
Pteropodidae	Little collared fruit bat	*Myonycteris torquata*	*Hepatocystis perronae* (Landau & Adam 1971)
Vespertilionidae	Large-footed mouse-eared bat	*Myotis adversus*	*Cryptosporidium tyzzeri* (Morgan *et al*. 1999)
Vespertilionidae	Alcathoe myotis	*Myotis alcathoe*	*Babesia canis canis* (Hornok *et al*. 2015)
Vespertilionidae	California myotis	*Myotis californicus*	*Eimeria californicus* (Duszynski *et al*. 1999a)
Vespertilionidae	Large myotis	*Myotis chinensis*	*Toxoplasma gondii* (Qin *et al*. 2014)
Vespertilionidae	Western small-footed bat	*Myotis ciliolabrum*	*Eimeria* sp. (Scott & Duszynski 1997)
Vespertilionidae	Western small-footed bat	*Myotis ciliolabrum*	*Cryptosporidium parvum* (Kváč *et al*. 2015)
Vespertilionidae	Western small-footed bat	*Myotis ciliolabrum*	*Eimeria rioarribaensis* (Duszynski & Barkley 1985)
Vespertilionidae	Daubenton's bat	*Myotis daubentonii*	*Bioccala murinus* (Gardner & Molyneux 1988)
Vespertilionidae	Daubenton's bat	*Myotis daubentonii*	*Babesia canis canis* (Hornok *et al*. 2015)
Vespertilionidae	Pond bat	*Myotis dasycneme*	*Besnoitia besnoiti* (Hornok *et al*. 2015)
Vespertilionidae	Gleaning myotis	*Myotis evotis*	*Eimeria evoti* (Duszynski *et al*. 1999a)
Vespertilionidae	Malagasy mouse-eared bat	*Myotis goudoti*	*Bioccala murinus* (Megali *et al*. 2011)
Vespertilionidae	Malagasy mouse-eared bat	*Myotis goudoti*	*Haemosporidia* sp. (Duval *et al*. 2007)
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Eimeria* sp. (Scott & Duszynski 1997)
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Eimeria catronensis* (Seville & Gruver 2004)
Vespertilionidae	Mouse-eared bat	*Myotis myotis*	*Bioccala murinus* (Dionisi 1899)
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Babesia vesperuginis* (Gardner & Molyneux 1987)
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	Unidentified coccidian (Gruber *et al*.1996)
Vespertilionidae	Natterer's bat	*Myotis natterei*	*Bioccala murinus* (Dionisi 1899)
Vespertilionidae	Black myotid bat	*Myotis nigricans*	*Bioccala deanei* (Garnham *et al*. 1971)
Vespertilionidae	Black myotid bat	*Myotis nigricans*	*Eimeria nigricani* (Duszynski *et al*. 1999b)
Vespertilionidae	Black myotid bat	*Myotis nigricans*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	*Eimeria kunmingensis* (reviewed by Seville & Gruver 2004)
Vespertilionidae	Rickett's big-footed bat	*Myotis ricketti*	*Toxoplasma gondii* (Qin *et al*.2014)

Vespertilionidae	Northern myotis	*Myotis septentrionalis*	*Eimeria catronensis* (McAllister *et al*. 2014)
Vespertilionidae	Northern myotis	*Myotis septentrionalis*	*Eimeria tumlisoni* (McAllister *et al*. 2012)
Vespertilionidae	Indiana bat	*Myotis sodalist*	Unidentified renal coccidian (Gruber *et al*.1996)
Vespertilionidae	Indiana bat	*Myotis sodalist*	*Klossiella* (Kusewitt *et al*. 1977)
Vespertilionidae	Long-legged myotis	*Myotis volans*	*Eimeria californicensis* (Seville & Gruver 2004)
Vespertilionidae	Yuma myotis	*Myotis yumanensis*	*Eimeria* sp. (Scott & Duszynski 1997)
Pteropodidae	Veldkamp's dwarf epauletted bat	*Nanonycteris veldkampii*	*Hepatocystis* (Schaer *et al*. 2013)
Nycteridae	Common noctule	*Noctalus noctula*	*Toxoplasma gondii* (Cabral *et al*. 2013)
Nycteridae	Common noctule	*Nyctalus noctula*	*Bioccala murinis* (Megali *et al*. 2011)
Nycteridae	Common noctule	*Nyctalus noctula*	Unidentified renal coccidian (Gruber *et al*. 1996)
Nycteridae	Common noctule	*Nyctalus noctula*	*Babesia canis canis* (Hornok *et al*. 2015)
Nycteridae	Bate's slit-faced bat	*Nycteris arge*	*Nycteria erardi* (Rosin *et al*. 1978)
Nycteridae	Large slit-faced bat	*Nycteris grandis*	*Nycteria* species (Schaer *et al*. 2015)
Nycteridae	Large-eared slit-faced bat	*Nycteris macrotis*	*Nycteria* species (Schaer *et al*. 2015)
Nycteridae	Dwarf slit-faced bat	*Nycteris nana*	*Nycteria houini* (Rosin *et al*. 1978)
Nycteridae	Egyptian slit-faced bat	*Nycteris thebaica*	*Nycteria medusiformis* (Garnham & Heisch 1953)
Molossidae	Broad-eared bat	*Nyctinomops laticaudatus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Molossidae	Big free-tailed bat	*Nyctinomops macrotis*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Vespertilionidae	Tri-colored bat	*Perimyotis subflavus*	*Eimeria mcdanieli* (McAllister *et al*. 2014)
Vespertilionidae	Tri-colored bat	*Perimyotis subflavus*	*Eimeria heidti* (McAllister *et al*. 2011)
Vespertilionidae	Tri-colored bat	*Perimyotis subflavus*	*Eimeria macyi* (Wheat 1975)
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus hastatus*	*Toxoplasma gondii* (Cabral *et al*. 2013)
Vespertilionidae	Himalayan pipestrelle	*Pipistrellus javanicus*	*Eimeria* sp. (Duszynski 1997)
Vespertilionidae	Himalayan pipestrelle	*Pipistrellus javanicus*	*Toxoplasma gondii* (Yuan *et al*. 2013)
Vespertilionidae	Kuhl's pipistrelle	*Pipistrellus kuhlii*	*Eimeria chiropteri* (Alyousif *et al*. 1999)
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Babesia vesperuginis* (Gardner & Molyneux 1987)
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Crytoptosporideum* genotype IV (Kváč *et al*. 2015)
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	Unidentified coccidians
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Toxoplasma gondii* (Dodd *et al*. 2014)
Vespertilionidae	Soprano pipistrelle	*Pipistrellus pygmaeus*	*Babesia canis canis* (Hornok *et al*. 2015)
Vespertilionidae	Soprano pipistrelle	*Pipistrellus pygmaeus*	*Toxoplasma gondii* (Dodd *et al*. 2014)
Phyllostomidae	Geoffroy's rayed bat	*Platyrrhinus lineatus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Molossidae	Brown mastiff bat	*Promops nasutus*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Pteropodidae	Greater musky fruit bat	*Ptenochirus jagori*	*Cryptosporidium* genotype II (Murakoshi *et al*.2016)
Pteropodidae	Black flying fox	*Pteropus alecto*	*Johnsprentia copema* (Landau *et al*. 2012b)
Pteropodidae	Black flying fox	*Pteropus alecto*	*Sprattiella alecto* (Landau *et al*. 2012b)

(*Continued*)

TABLE 11.1 (Continued)

Bat family	Bat common name	Bat species	Apicomplexan (Reference)
Pteropodidae	Black flying fox	*Pteropus alecto*	*Hepatocystis levinei* (Landau *et al*. 1985)
Pteropodidae	Black flying fox	*Pteropus alecto gouldi*	*Hepatocystis pteropi* (Breinl 1911)
Pteropodidae	Spectacled flying fox	*Pteropus conspicillatus*	Unidentified *Toxoplasma* sp.
Pteropodidae	Variable flying fox	*Pteropus hypomelanus*	*Hepatocystis* species (Megali *et al*. 2011)
Pteropodidae	Gray-headed flying fox	*Pteropus poliocephalis*	*Hepatocystis levinei* (Landau *et al*. 1985)
Pteropodidae	Little red flying fox	*Pteropus scapulatus*	Unidentified *Toxoplamsa* sp.
Rhinolophidae	Intermediate horseshoe bat	*Rhinolophus affinis*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Rhinolophidae	Halcyon horseshoe bat	*Rhinolophus alcyone*	*Nycteria* sp. (Schaer *et al*. 2013)
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	*Toxoplasma gondii* (Qin 2014)
Rhinolophidae	Philippine forest horseshoe bat	*Rhinolophus inops*	*Cryptosporidium* genotype V (Murakoshi *et al*. 2016)
Rhinolophidae	Hildebrandt's horseshoe bat	*Rhinolophus hildebrandti*	*Nycteria congolensis* (Krampitz *et al*. 1960)
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	*Eimeria hessei* (Alfonso *et al*. 2014)
Rhinolophidae	Lander's horseshoe bat	*Rhinolophus landeri*	*Nycteria congolensis* (Schaer *et al*. 2015)
Rhinolophidae	Blyth's horseshoe bat	*Rhinolophus pusillus*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	*Cryptosporidium* genotype I (Wang *et al*. 2013)
Rhinolophidae	Chinese rufous horseshoe bat	*Rhinolophus sinicus*	*Cryptosporidium* genotype II (Wang *et al*. 2013)
Rhinolophidae	None	*Rhinolophus sylvestri*	*Nycteria gabonensis* (Rosin *et al*. 1978)
Rhinolophidae	Horseshoe bats	*Rhinolophus* sp.	*Nycteria krampitzi* (Rosin *et al*. 1978)
Pteropodidae	Geoffroy's rousette	*Rousettus amplexicaudatus*	*Eimeria* sp. (Murakoshi *et al*.2016)
Pteropodidae	Straw-colored fruit bat	*Roussetus aegyptiacus leachi*	*Plasmodium rousseti* (Van Riel *et al*. 1951)
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	*Toxoplasma gondii* (Yuan *et al*. 2013)
Pteropodidae	Leschenault's rousette	*Rousettus leschenaultia*	*Cryptosporidium* genotype II (Wang *et al*. 2013)
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Vespertilionidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Toxoplasma gondii* (Cabral *et al*. 2014)
Vespertilionidae	Pocketed free-tailed bat	*Tadarida femorosacca*	*Eimeria tadarida* (Duszynski *et al*. 1988)
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	*Eimeria andamanensis* (Mandal & Nair 1973)
Emballonuridae	Black-bearded tomb bat	*Taphozous melanopogon*	*Toxoplasma gondii* (Jiang *et al*. 2014)
Emballonuridae	Egyptian tomb bat	*Taphozous perforatus*	*Nycteria medusiformis* (Morsy *et al*. 1987)
Molossidae	Blunt-eared bat	*Tomopeas ravus*	*Eimeria tomopea* (Duszynski & Barkley 1985)
Rhinolophidae	Trouessart's trident bat	*Triaenops furculus*	*Haemosporidia* sp. (Duval *et al*. 2007)
Phyllostomidae	Brown tent-making bat	*Uroderma magnirostrum*	*Eimeria* sp. (Duszynski *et al*. 1999b)
Vespertilionidae	Parti-colored bat	*Vespertilio murinus*	*Bioccala murinus* (Dionisi 1899)
Vespertilionidae	Serotine bat	*Vespertilio serotinus*	*Toxoplasma gondii* (Cabral *et al*. 2013)
Vespertilionidae	Asian parti-colored bat	*Vespertilio superaus*	*Toxoplasma gondii* (Yuan *et al*. 2013)

11.2 ORDER HAEMOSPORIDA

The life cycle of the intracellular parasites of the order Haemosporida includes macro- and microgamonts which develop independently. The microgamont produces eight flagellated microgametes. Fertilization of the macrogamete by a microgamete in the invertebrate definitive host produces a zygote, the oocyte. This will undergo asexual reproduction using multiple fission in a process known as sporogony. The resulting sporozoites serve as the infectious form for the intermediate vertebrate host and are naked. Their transmission is via the bite of haemophagous insects (Roberts & Janovy Jr 2006). The order Haemosporida is composed of over 550 species which are divided into four families: Garniidae, Haemoproteidae, Leucocytozoidae, and Plasmodiidae. The latter includes five members of the *Plasmodium* genus which infect humans (*Plasmodium falciparum, P. vivax, P. ovale, P. malariae*, and *P. knowlesi)*. These cause malaria which may be highly pathologic and potentially fatal. Other *Plasmodium* species infect animals, including birds. These parasites, especially *P. falciparum*, have been, and remain, one of the leading infectious causes of death, especially among the young, in those regions of the world still inhabited by their *Anopheles* mosquito vectors (their definitive host).

The *Plasmodium* genus of Haemosporida is recognized by their unique ability to replicate asexually in their intermediate host's erythrocytes by schizogony to produce merozoites. Their progeny, in turn, burst out of the host cell to infect the next set of host erythrocytes. The repeated rounds of invasion and rupture of erythrocytes and the formation of toxic by-products of hemoglobin are the primary causes of pathology. Other Haemosporida genera use alternative tissues for asexual reproduction. A minority of the *Plasmodium* schizonts infecting erythrocytes will form male and female gametocytes, to be ingested by an arthropod definitive host during a blood meal. The female gametocyte produces a single large macrogamete (similar to an egg), while the male gametocyte produces many small microgametes (similar to sperm). Within the arthropod definitive host, a microgamete fertilizes the macrogamete to form an oocyte. This is transformed into the motile ookinete that is able to penetrate the wall of the arthropod's gut and develop on the wall's exterior. Next, another round of asexual reproduction produces many sporozoites. This form of the *Plasmodium* parasite makes its way to the arthropod's salivary glands and is transferred to the intermediate vertebrate host during the arthropod's blood meal. It is believed that the haemosporidian parasites which lack schizogony in the blood are generally less pathogenic than those which do undergo this process in erythrocytes (reviewed in Schaer *et al*. 2015).

A hallmark of haemosporidian parasites in general is an obligate host switch between an arthropod definitive host and one of a wide assortment of vertebrate intermediate hosts, which include mammals, birds, and reptiles. Of the known haemosporidian genera, bats are the exclusive mammalian host for seven, with the exception of *Plasmodium* and *Hepatocystis*, which both infect a wide range of intermediate hosts as well as bats, and the *Rayella* genus (reported only in flying squirrels) (reviewed in Schaer *et al*. 2015).

11.2.1 Invertebrate hosts of Haemosporida

Haemosporidians utilize at least seven families of hematophagous dipteran insects as vectors and the association between the specific insect vectors and haemosporidia is stronger than that between the parasites and their vertebrate intermediate hosts (reviewed in

Megali *et al*. 2011). Nycteribiidae bat flies are the major vectors for haemosporidia species. These flies are unique, serving as the only known wingless vectors of malaria. The colonial nature of many bat species, leading to close contact between its members, may help facilitate horizontal transfer of the flies between bat hosts. Unlike the brief encounters of mosquitoes with their hosts, Nycteribiidae are permanent ectoparasites, with males and female flies feeding exclusively on host blood (reviewed by Megali *et al*. 2011). *Myotis myotis* and *Nyctalus noctula* bats, however, host flies of the genus *Penicillidia*, a vector of *Polychromophilus* parasites in Israel and Africa (Landau *et al*. 1980b).

11.2.2 Bat hosts of Haemosporida

Thirteen species of bats from the Upper Guinean forest ecosystem host at least four genera of haemosporidian parasites: *Plasmodium, Polychromophilus, Nycteria*, and *Hepatocystis* (Schaer *et al*. 2013). Overall prevalence of haemosporidians among these bats was 40%.

In a study of seven bat families in Madagascar and Cambodia, haemosporidian species were only found in the blood of three bat families: Hipposideridae, Vespertilionidae, and Megadermatidae (Duval *et al*. 2007). Work in Australia has found four types of haemosporidia in *Pteropus alecto* bats: *Hepatocystis levinei* with its hepatic schizonts, also present in *Pteropus poliocephalis* bats; hepatic schizonts of *Johnsprentia copemani* parasites; blood gametocytes of *Hepatocystis* species; and blood gametocytes of *Sprattiella alecto* parasites (Landau *et al*. 2012b). *Polychromophilus, Bioccala, Johnsprentia*, and *Sprattiellai* haemosporidian species all form schizonts in the reticuloendothelial system while *Plasmodium, Nycteria, Hepatocystis*, and *Biguetiella* produce hepatic schizonts. See Landau *et al*. (2012a) for an excellent review of the morphology and other characteristics of these genera of blood parasites as well as a list of haemosporidians infecting bats that are found in Table 11.1 but are not otherwise mentioned specifically in the text.

11.2.2.1 Plasmodium species and bats Two *Plasmodium* species were detected at high prevalence exclusively in the African frugivorous bats *Myonycteris angolensis* and *Roussettus smithi*, infected by *Plasmodium voltaicum* parasites, and the insectivorous bat *Hipposideros cyclops*, infected by *Plasmodium cyclopsi* (Van Der Kaay 1964; Schaer *et al*. 2013). The *Plasmodium* species of bats are similar to those found in rodents.

11.2.2.2 Polychromophilus species and bats The *Polychromophilus melanipherus* parasites found in insectivorous *Miniopterus villiersi* bats are part of the same clade as *P. melanipherus* samples from *Miniopterus schreibersii* bats. Members of the *Polychromophilus* genus appear to be more closely related to avian malaria parasites than to those of mammals (Duval *et al*. 2012). Interestingly, in a cave in Gabon, *P. melanipherus* only infected *Miniopterus inflatus* even though *Hipposideros gigas, Hipposideros caffer*, and *Coleura afra* bats lived in very close proximity and the bat fly vector, the nycteribeciid *Penicillidia fulvida*, is also found on *H. caffer* and *C. afra* bats. Additionally, *Polychromophilus* parasites have been found to infect *P. fulvida* flies in Gabon (Landau *et al*. 1980b).

In a study of the characteristics of infection of Swiss *Myotis daubentonii* bats by *Polychromophilus murinus*, juvenile bats had a much higher parasite load than other bats of other age groups, yet without any observed direct physiological effect of infection

(Witsenburg *et al*. 2014). This tendency to infect younger bats may be due to the tendency of bat ectoparasites to move in mass onto the pups shortly after birth, thus introducing the haemosporidia into neonatal bats during their very early stages of development, before they have developed a fully functional adaptive immune system and thus enabling high levels of parasitemia. The reproductive cycles of the bats and their ectoparasites are synchronized, so that the newly emerged ectoparasite offspring receive an abundant number of haemosporidia at an early age as well. In subadult animals, body condition and intensity of infection were negatively correlated. Surprisingly, neither body temperature (as a measure of fever) nor hematocrit (a proxy for the presence of anemia) correlates with infection intensity (Witsenburg *et al*. 2014). The lack of anemia may be in part due to the lack of this group of parasites' asexual reproduction in bats' erythrocytes, leading to the absence of destruction of bat red blood cells, unlike that which occurs during *Plasmodium* infections.

Two insectivorous bats, *Pipistrellus grandidieri* and *Neoromicia capensis*, are infected with by haemosporidia very similar to *P. murinus* derived from European vespertilionid bats (Schaer *et al*. 2013).

11.2.2.3 Nyceteria species and bats *Nycteria* species parasites have been detected in the blood of two horseshoe bats, *Rhinolophus alcyone* and *Rhinolophus landeri*. *Nycteria* is believed to be a sister clade to the mammalian *Plasmodium/Hepatocystis* clade. This study's phylogenetic analysis indicates that bats may have been *Plasmodium*'s first mammalian hosts, later followed by the infection of rodents and primates. Only bats from the genera *Rhinolophus* (Rhinolophidae family) and *Nycteris* (Nycteridae family) appear to be infected with *Nycteria* parasites, while all tested members of the bat families Hipposideridae ($n=56$), Emballonuridae ($n=4$), and Megadermatidae ($n=33$) tested negative for *Nycteria* infection (Schaer *et al*. 2015). At least six *Nycteris* species of slit-faced bats appear to be infected: *Nycteris arge* by *Nycteria erardi*; *Nycteris capensis* by *Nycteria medusiformis*; *Nycteris grandis* by *Nycteris macrotis* as well as by another, unidentified *Nycteria* species; *Nycteris nana* by *Nycteria houini*; and *Nycteris thebaica* by *M. medusiformis* (review by Sangster *et al*. 2012).

11.2.2.4 Hepatocystis species and bats The *Hepatocystis* species in Guinean bats have a high degree of diversity and prevalence in several species of fruit bats – *Epomophorus gambianus, Epomops buettikoferi, Hypsignathus monstrosus, Micropteropus pusillus, Myonycteris leptodon*, and *Nanonycteris veldkampii*. The *Hepatocystis* clade appears to have been derived from mammalian *Plasmodium* parasites and rarely causes disease.

11.2.2.5 Bioccala species and bats While most haemosporidian species occur only in tropical regions of Asia and Africa, they have also been found to parasitize temperate zone bats in Europe and the Americas. *Bioccala murinus* was found in four bat species and in its insect vector, the bat fly *Nycteribia kolenatii*. Prevalence of parasite infection varied widely: 4% in *M. myotis* ($n=47$), 7% in *N. noctula* ($n=15$), 11% in *Eptesicus serotinus* ($n=18$), and 51% in *M. daubentoni* ($n=127$) (Megali *et al*. 2011).

11.2.2.6 Hepatozoon species and bats *Hepatozoon* DNA sequences were amplified from livers of the Southeast Asian bat *Hipposideros cervinus* from Sarawak in Malaysian Borneo (Pinto *et al*. 2013). The genus *Hepatozoon* consists of intracellular

parasites that infect a wide range of tetrapods in addition to the parasites' definitive hosts, hematophagous arthropods. The tetrapods are primarily infected by ingestion of the arthropods. *Hepatozoon* infection leads to skeletal muscle degeneration and atrophy as well as recurrent fever, lethargy, depression, weight loss, stiffness, and lameness in dogs (Paludo *et al*. 2005).

11.3 ORDER PIROPLASMIDA

Parasites of this order may appear as piriform, round, ameboid, or rod-shaped. They lack oocysts and spores. Like members of the order Haemosporida, they parasitize erythrocytes. Piroplasmida includes the genus *Babesia* which causes a severe, malaria-like disease in immunocompromised humans. The presence of undetected *Babesia* in our blood supply, as well as increasing numbers of immunosuppressed people, makes members of this genus potentially significant emerging health concerns in the near future (reviewed by Beltz 2011). The order Piroplasmida also includes the genus *Theilaria*, some of whose species cause serious disease in cattle, but has not been reported in bats.

11.3.1 *Babesia* species and bats

Babesia vesperuginis is present in blood of the British bats *Pipistrellus pipistrellus* and *Myotis mystacinus* (Gardner & Molyneux 1987). Naturally or experimentally infected *P. pipistrellus* had lowered blood hemoglobin levels, increased numbers of reticulocytes (immature red blood cells) and white blood cells, and enlarged spleens, suggesting that this parasite may be pathogenic for bats. Blood samples from two of 168 Columbian leaf-chinned bats (*Mormoops megalophylla*) were also infected by *B. vesperuginis* (Marinkelle 1996). Approximately 18% of these bats' red blood cells were infected and their spleens were enlarged to eight to nine times the size of those of uninfected bats.

In Europe, *Babesia canis canis*, which typically infects canines, was identified in bat fecal samples from five Hungarian bat species (100%). This study also found these parasites in fecal samples from bats in the Netherlands that had a 99% homology to *Besnoitia besnoiti*, an emerging apicomplexan of cattle in Europe that is responsible for a chronic and debilitating disease (Gazzonis *et al*. 2014). Of note, areas with a high prevalence of apicomplexans in dogs also had a higher prevalence of infected bat feces than otherwise. The following bat species tested positive for *B. canis canis*: *N. noctula, M. daubentonii, Pipistrellus pygmaeus*, and *Myotis alcathoe*. Fecal material from the pond bat (*Myotis dasycneme*), by contrast, contained *B. besnoiti* (Hornok *et al*. 2015). The fact that these blood apicomplexans were detected in fecal samples suggests that they passed from the gut vascular system into the intestinal tract (Hornok *et al*. 2015). Together, these studies indicate that *Babesia* is found in several distinct areas of Europe and may have a much larger distribution in bats than was previously believed.

11.3.2 Other Piroplasmida in bats

Klossiella sporonts have been reported in the kidneys of *Myotis sodalist* in the US (Kusewitt *et al*. 1977). *Klossiella killicki* coccidians have also been reported to infect the renal system of *H. caffer* bats in Africa (Boulard 1975). This unusual coccidian completes

its entire cycle in the kidney of its bat host rather than requiring two host species in order to complete its life cycle (Wünschmann *et al*. 2010). Coccidian sporogony typically requires oxygen and thus exposure to external air.

Unidentified coccidians have been found in several bat host groups: *P. pipistrellus, M. mystacinus, Myotis nattereri*, and *N. noctula* (Gruber *et al*. 1996). The affected bats were found dead and had suffered from severe renal coccidiosis. The dead bats included males and females, adults and juveniles. The kidney surfaces of these bats contained numerous irregularly distributed and somewhat indented white foci. In addition to the infection of the kidney epithelium, the kidney's cystic tubules were dilated and almost completely filled with asexual and sexual coccidian developmental stages, including schizonts, macrogamonts, and microgamonts. It remains to be determined whether the coccidians are bat pathogens or commensal organisms since the dead bats had adequate fat deposits and appeared to have been in good health. No inflammation was detected, suggesting that the host immune system may not have been activated by this infection. In a separate study of *M. sodalis*, only asexual stages of *Babesia* species were found without gross dilation of infected renal tubules (Gruber *et al*. 1996); however, different bat species were examined, perhaps explaining the difference in pathology. This may indicate that renal infection and damage may be species-specific. Differences in pathology might also be a function of the type of coccidian involved.

11.4 ORDER EIMERIIDA

The Eimeriida order of Apicomplexans includes approximately 1700 members and is the most species-rich genera of all of the coccidians. Its members infect virtually all groups of vertebrates. Some of the traits of this order include independent development of macro- and microgamonts, the latter of which produces multiple microgametes; a zygote that is immobile, and sporozoites that are enclosed in a sporocyst within an oocyte (Roberts & Janovy Jr 2006). Identification of eimerians has often relied heavily upon oocyte morphology, but has recently begun to include molecular analysis.

Eimeriida genera include several parasites of humans and domestic animals that pose significant health problems and economic loss. These include *Toxoplasma, Eimeria, Isospora*, and *Sarcocystis*. *Toxoplasma* causes severe health risks to developing fetuses and immunocompromised people, as described more fully below. *Eimeria tenella* inhabits the intestinal epithelium of chickens and kills a large number of young birds. Other eimerians infect additional agricultural animals, including turkeys, ducks, cattle, sheep, and pigs. *Isospora belli* rarely infects humans, but may cause persistent diarrhea or death in people with AIDS. *Sarcocystis* species infect up to 50% of adult cattle, pigs, and sheep. Some of these species cause significant loss of weight, anemia, and abortion in pregnant animals (Roberts & Janovy Jr 2006).

11.4.1 *Toxoplasma gondii* and bats

Approximately one third of the world's human population has antibodies to *T. gondii*, indicating at least some exposure to the parasite. *Toxoplasma* species use two hosts in their life-cycle – a definitive host in which sexual reproduction occurs and an intermediate host in which asexual reproduction occurs. Transmission of *T. gondii* occurs by the

intermediate hosts ingesting tissue cysts, raw infected meat, or oocysts shed in the feces of the definitive host into the environment. While cats are the only known definitive host, intermediate hosts include many vertebrates, including humans, other mammals, and birds. Transplacental infection also occurs in women infected during pregnancy, often accompanied by severe neural damage to the developing fetus or miscarriage. Immunocompromised adults, especially HIV-positive individuals, are also susceptible to serious neurological disease.

Infection with *T. gondii* led to systemic toxoplasmosis and death in two juvenile, captive flying foxes: the spectacled flying fox (*Pteropus conspicillatus*) and the little red flying fox (*Ptretopus scapulatus*) from Australia (Sangster *et al*. 2012). The parasite was found in the brain and the heart. The infected animals had respiratory distress and loss of pectoral muscle mass. One bat appeared to have neurological disease as well. Some other species of flying foxes may also act as intermediate hosts

The presence of antibodies to *T. gondii* in Brazilian bats was first reported in 1969 with the isolation of the parasite from the greater spear-nosed bat, *Phyllostomus hastatus* (reviewed by Cabral *et al*. 2014). A much later study in a large forested park in extreme northern Brazil found that 21.5% of the tested bats produced IgG anti-*T. gondii* antibodies. The positive bats were: the great fruit-eating bat, *Artibeus lituratus*; the short-tailed fruit bat, *Carollia perspicillata*; and Pallas's long-tongued bat, *Glossophaga soricina* (Fournier *et al*. 2014). These bats are either frugivorous or omnivorous. Of great importance for human health, many of the cat definitive hosts for *T. gondii* residing in this forest produced these antibodies as well. By contrast, in the very large metropolitan area of São Paulo city, Brazil, *T. gondii* was detected in only a small number of an insectivorous bat, the velvety free-tailed bat (*Molossus molossus*), and in a hematophagous bat, the common vampire bat (*Desmodus rotundus*) (Cabral *et al*. 2013). Another study by the same investigators of bats in São Paulo city found that 32.62% of tested animals ($n=616$) produced anti-*T. gondii* antibodies, but these were primarily of low titer. Recently, the number of bats in urbanized areas of Brazil has been increasing, perhaps due to changes in their previous environment together with the availability of new habitats with abundant food and lower numbers of bat predators.

In Kazakhstan in southern and western Asia, *T. gondii* was isolated from the insectivorous common noctule (*Noctalus noctule*) and the serotine bat (*E. serotinus*) (reviewed by Cabral *et al*. 2013). When the percentage of bats seropositive to *T. gondii* was measured in animals from four distinct regions of China, anti-*T. gondii* antibodies were detected in 26.5% of the carnivorous greater false vampire bat (*Megaderma lyra*; ($n=68$), 13.6% of the frugivorous Leschenault's rousette (*Rousettus leschenaultia*) ($n=88$), 13.6% of the greater short-nosed fruit bat (*Cynopterus sphinx*) ($n=22$), 20% of the insectivorous Asian parti-coloured bat (*Vespertilio superaus*) ($n=20$), and 15.8% of the insectivorous Himalayan pipistrelle (*Pipistrellus javanicus*) ($n=19$). Four of the five bat species had relatively high antibody titers of at least 200 (Yuan *et al*. 2013). No geographical differences were seen in prevalence of infection. The higher rate of infection in *M. lyra* may be a function of their diet, since they primarily feed on mice and sparrows, both of which may serve as intermediate hosts of *T. gondii*, while the other four bat groups are insectivorous or frugivorous bats (Yuan *et al*. 2013).

Approximately 7.9% of the Chinese human population is seropositive for *T. gondii*, making this parasite a significant health concern. In a recent study of ten species of Chinese bats in four other Chinese provinces, liver samples from eight bat species were

found to be PCR-positive for the *T. gondii* B1 gene: 3.6% of the insectivorous greater tube-nosed bat (*Murina leucogaster*) ($n=222$), 7.2% of the insectivorous large myotis (*Myotis chinensis*) ($n=139$), 3.6% of the Rickett's big-footed myotis (*Myotis ricketti*) ($n=56$), 14.0% of the insectivorous greater horseshoe bat (*Rhinolophus ferrumequinum*) ($n=43$), 1.9% of the frugivorous *C. sphinx* ($n=54$), 10.0% of the frugivorous *R. leschenaultia* ($n=30$), and 11.9% of the insectivorous intermediate roundleaf bat (*Hipposideros larvatus*) ($n=67$). Incidence of infection appears to be greater in bats from the southern provinces than in those from northern China (Qin *et al*. 2014). The 38 parasite isolates which were completely genotyped belonged to either ToxoDB Genotype #10 or to ToxoDB Genotype #9. Both genotypes had been reported in *Microtus fortis* bats in Jilin province, China (Zhang *et al*. 2014), along with domestic animals and humans. ToxoDB #9 is also present in Vietnam and Sri Lanka. A 2013 study of 550 bats from six species in Myanmar found that 29.3% of the tested bats in five of the species were PCR-positive for *T. gondii* (Sun *et al*. 2013). The infected bat species are as follows: 38.8% of the insectivorous Japanese long-fingered bat (*Miniopterus fuliginosus*) ($n=353$), 4.9% of insectivorous *R. ferrumequinum* ($n=162$), 90% of insectivorous *M. chinensis* ($n=10$), 12.5% of the insectivorous *Hipposideros armiger* ($n=8$), and 100% of the carnivorous *M. lyra* ($n=6$) (Sun *et al*. 2013). A separate study in southern China used PCR to detect the presence of *T. gondii* DNA from 608 bats representing 12 species from two geographical locations (Jiang *et al*. 2014). From the bats collected from Guangxi, 20.3% were PCR-positive ($n=103$), including 45.8% of the *Taphozous melanopogon* studied. From the bats collected from Yunnan, 6.7% were found to be positive for *T. gondii* DNA ($n=475$). Different locations may therefore differ in the incidence of infection. The twelve infected species from both locations were as follows: 8.3% of the carnivorous Stoliczka's trident bat (*Aselliscus stoliczkanus* ($n=120$), 18.6% of the insectivorous *M. chinensis* ($n=59$), 3.1% of the insectivorous Blyth's horseshoe bat (*Rhinolophus pusillus*) ($n=32$), 1.2% of the insectivorous great roundleaf bat (*H. armiger*) ($n=81$), 3.6% of the insectivorous Fulvus roundleaf bat (*Hipposideros fulvus*) ($n=28$), 7.5% of the insectivorous *Rhinolophus affinis* ($n=53$), the insectivorous *R. pusillus*, the insectivorous *H. larvatus*, and the insectivorous black-bearded tomb bat (*T. melanopogon*), 7.1% of the frugivorous *C. sphinx* ($n=14$), and 6.7% of *Eonycteris spelaea* ($n=45$) (Jiang *et al*. 2014). Both frugivorous and insectivorous bats, therefore, are infected by *T. gondii* of two genotypes (ToxoDB#9 and #10) as reported by Qin *et al*. (2014).

A study in the UK used PCR to examine the prevalence of *T. gondii* DNA in two pipistrelle species, the common pipistrelle (*P. pipistrellus*) and the Soprano pipistrelle (*P. pygmaeus*) (Dodd *et al*. 2014). Parasite prevalence in *P. pipistrellus* was 9.9% ($n=71$), while that of *P. pygmaeus* was 16.7% ($n=6$). No significant differences were observed between infected and uninfected animals in body condition index (weight/forearm length ratio.) Since all species of British bats are insectivorous, it is possible that infection occurred by drinking water containing oocytes and then passed on transplacentally from mother to offspring (Dodd *et al*. 2014). In France, *T. gondii* was found in the brain of a *Myotis bechsteinii* and the Daubenton's myotis (*M. daubentonii*), both insectivorous as well (reviewed by Cabral *et al*. 2014).

Kidneys of *E. fuscus* bats contain many life-cycle stages of a small coccidian parasite (*Nephroisospora eptesicinov*). Infection of these bats typically leads to generally mild, focal or multifocal, well-demarcated cortical renal lesions (Wünschmann *et al*. 2010). This coccidian is unusual since all of the stage of its life cycle occur in the kidneys

of a single host. This parasite is closely related to pathogenic *Toxoplasma, Besnoitia*, *Hammondia*, and *Neospora* apicomplexan species found in other mammals (Wünschmann *et al*. 2010). *Besnoitia* infections lead to a chronic, debilitating condition that is responsible for significant economic losses in infected cattle, while *Neospora caninum* causes an illness similar to *Toxoplasma* in young dogs, resulting in paralysis and death, abortions in cattle and sheep, and more generalized nervous system disorders in kittens (Roberts & Janovy Jr 2006).

11.4.2 *Eimeria* species and bats

A study of coccidians in the Southwest US and Sonora, Mexico, detected a novel species of sporulated oocysts in the feces of a *Tadarida femorosacca* bat ($n=18$), but none of the tested *T. brasiliensis* ($n=12$). The parasite species was tentatively named *Eimeria tadarida* (Duszynski *et al*. 1988). A prior study in Bolivia in the mid-1980s described the sporulated oocysts and ovoid sporocysts of *Eimeria nigricani* from feces of the black myotid bat (*Myotis nigricans*) as well as a previously unknown species of *Eimeria* in a tent-making bat (*Uroderma magnirostrum*) (Duszynski *et al*. 1999b). A separate study by the same group described the ovoid sporocysts within sporulated oocysts in the feces of crevice bats, *Tomopeas ravus*, from Peru. This new species was named *Eimeria tomopea* (Duszynski & Barkley 1985). Another study by this group in Bolivia, Mexico, and Southern California described *Eimeria* species in feces of several species of bats (Scott & Duszynski 1997). Ellipsoidal sporulated oocysts containing football-shaped sporocysts were found in 11% of the fecal samples from *Myotis lucifugus* ($n=27$) and *Myotis yumanensis* ($n=70$). This study also described a second new species of eimerian from 6 to 8% of feces from *M. yumanensis* ($n=70$) and *Myotis ciliolabrum* ($n=12$), as well as an eimerian from the pallid bat (*Antrozous pallidus*) ($n=85$) whose morphology was very similar to *Eimeria arizonensis* (Scott & Duszynski 1997), but was later shown by molecular analysis of plastid 23S rDNA and nuclear 18S rDNA sequences to be *Eimeria antrozoi* (Zhao *et al*. 2001).

In the US, oocysts of *Eimeria tumlisoni* were detected in 75% of the fecal samples of the northern myotis (*Myotis septentrionalis*) from Oklahoma and Arkansas ($n=4$) (McAllister *et al*. 2012*). Eimeria catronensis* was also found in *M. septentrionalis* bats in Oklahoma (McAllister *et al*. 2014). Eighteen percent of the fecal samples ($n=11$) from tri-colored bats (*Perimyotis subflavus*) from Arkansas contained *Eimeria mcdanieli* as well as *Eimeria heidti* (McAllister *et al*. 2014). *Eimeria macyi* oocytes were also found in and described in fecal samples from *P. subflavus* from Alabama in the southern US (Wheat 1975). *E. catronensis* were present in *M. lucifugus* and *Eimeria californicensis* bats and from a long-legged myotis (*Myotis volans*) from Wyoming in the western US (Seville & Gruver 2004). The same Wyoming study also recovered oocytes of *E. catronensis* from the feces of the silver-haired bat (*Lasionycteris noctivagans*). *E. californicensis* had been previously reported in the California myotis (*Myotis californicus*) in California, on the country's southern Pacific coast (Duszynski *et al*. 1999a). This study also detected and described *Eimeria evoti* from the gleaning myotis (*Myotis evotis*) and *Eimeria rioarribaensis* from the western small-footed myotis (*M. ciliolabrum*) in New Mexico. In Arkansas, *E. catronensis* was found in the northern long-eared myotis (*M. septentrionalis*), and *Eimeria dowleri* and *Eimeria sealanderi* from eastern red bats (*Lasiurus borealis*) (Scott & Duszynski 1997; McAllister & Upton 2009; McAllister *et al*. 2014).

In the Eastern hemisphere, sporulated oocysts of the coccidian species *Eimeria chiropteri* were found in fecal material of *Pipistrellus kuhlii* in Saudi Arabia (Alyousif *et al*. 1999). *Eimeria levinei* was reported from the gland-tailed free-tailed bat (*Chaerephon bemmeleni*) from Liberia (Bray 1958) and *Eimeria andamanensis* was found in the black-bearded tomb bat (*T. melanopogon*) from India (Mandal & Nair 1973; reviewed by McAllister *et al*. 2012). A study of Philippine bats (Sangster *et al*. 2012) found that 15.6% tested positive for *Eimeria* species. *Eimeria* BE3 DNA sequences from *Scotophilus kuhlii* bats were classified with previously known bat and rodent clades; however, sequences derived from *C. brachyotis, E. spelaea, Rousettus amplexicaudatus*, and *R. inops* did not classify with other known eimerians. In Asia, *Eimeria kunmingensis* was detected in *M. ricketti* bats from China (reviewed by Seville & Gruver 2004).

A French study obtained a partial sequence of the 18S rRNA gene of *Eimeria hessei* in maternity roost feces from the lesser horseshoe bat (*Rhinolophus hipposideros*). This parasite was present in all eleven of the tested roosts. The eimerian's identity was confirmed by the oocytes' morphological characteristics (Afonso *et al*. 2014). Interestingly, *E. hessei* is phylogenically related to *Eimeria chobotari* and *Eimeria dipodomysis* of American rodents, specifically kangaroo rats (87% bootstrap support). Many of the other eimerians in bats also belong to clades which include eimerians of rodents, leading to the proposal that the parasites have at some point in time undergone lateral transfer between bats and rodents (Zhao *et al*. 2001).

11.5 ORDER ADELEIDA, *CRYTOPORIDIUM* SPECIES, AND BATS

Among other traits, the sporozoites of the *Adeleida* order of Apicomplexa are enveloped. This order includes the *Cryptosporidium* genus whose members may cause cryptosporidiosis, a sometimes severe illness, particularly in people who are immunocompromised, such as HIV-positive people. These parasites, however, may also lead to significant gastrointestinal disease among immunocompetent people. Six species of *Cryptosporidium* in humans have been associated with cryptosporidiosis: *Cryptosporidium hominis, Cryptosporidium parvum, Cryptosporidium meleagridis, Cryptosporidium viatorum, Cryptosporidium felis*, and *Cryptosporidium canis*. Several other species have been recently identified in humans, including *Cryptosporidium muris, Cryptosporidium suis, Cryptosporidium andersoni, Cryptosporidium ubiquitum*, and *Cryptosporidium cuniculus* (reviewed by Murakoshi *et al*. 2016). Infection in susceptible individuals leads to severe, persistent, and sometimes fatal diarrhea. A major outbreak due to fecal contamination of the water supply in Milwaukee in 1993 affected more than 400 000 people (Beltz 2011).

C. parvum is not a uniform species but instead is composed of numerous genotypes, including a "human" genotype found only in humans; a "cattle" genotype containing cattle isolates, five Australian mouse isolates, and a wildebeest isolate; and a "mouse" group that includes all Australian mouse isolates, all UK, several Spanish mouse isolates, and a bat isolate from New South Wales (Morgan *et al*. 1999). Four "bat" *Cryptosporidium* species have also been reported. New species of *Cryptosporidium* are defined on the basis of oocyst morphology, genetic characterization, and host specificity. New genotypes, by contrast, are based on sequencing differences at the SSU rRNA locus or on other molecular criteria.

A variety of cryptosopodian species are found in Australia and the Philippians. *Cryptosporidium tyzzeri*, a species similar to *C. parvum*, was isolated from fecal material of the large-footed mouse-eared bat (*Myotis adversus*) in Australia (Morgan *et al*. 1999). Intestinal samples of various Philippian bats were tested by PCR for the presence of cryptosporidian and eimerian DNA. Members of the fruit bat species *Cynopterus brachyotis* ($n=15$) and *E. spelaea* ($n=3$) were PCR-positive for both of these types of apicomplexans, *Ptenochirus jagori* ($n=12$) was positive for only *Cryptosporidium* DNA, while *R. amplexicaudatus* ($n=5$) was only positive for *Eimeria* DNA. Of the insectivorous bats, *S. kuhlii* ($n=1$) was positive for *Eimeria* DNA, while both *Cryptosporidium* and *Eimeria* were found in *Rhinolophus inops* intestinal tissue ($n=1$) (Murakoshi *et al*. 2016). Sangster *et al*. (2012) found that 8.8% of tested Philippian bats were positive for several *Cryptosporidium* species ($n=45$). *P. jagori* carried *Cryptosporidium* bat genotype II, while three additional previously unclassified *Cryptosporidium* were detected in *R. inops, C. brachyotis*, and *E. spelaea*. The authors proposed that these species be classified as novel bat Cryptosporidium genotype V, genotype VI, and genotype VII, respectively. Significantly, bat genotype V is associated with the human cryptosporidiosis clade and might be transmissible to humans (Sangster *et al*. 2012).

Smaller numbers of crytosporidians have been reported from other areas of the world. In China, a molecular study detected two new genotypes of *Cryptosporidium* in four species of bats. Two *Cryptosporidium* from *A. stoliczkanus* and *Rhinolophus sinicus* were placed into genotype I, while those from *R. leschenaultia, R. sinicus*, and *H. fulvus* were placed into genotype II (Wang *et al*. 2013).

In Europe, *C. parvum* belonging to the bat genotype IV was detected from *P. pipistrellus* in the Czech Republic (Kváč *et al*. 2015). In Oregon, in the western US, big brown bats (*E. fuscus*) were first found to have an intestinal infection with a cryptosporidial species in a study by Dubey *et al*. (1998) and, recently, these bats were found to carry a new member of the bat genotype III group (Kváč *et al*. 2015). Additionally, the latter study detected *C. parvum* in the western small-footed bat (*M. ciliolabrum*).

11.6 CONCLUSIONS

Members of the Coccidae class of the phylum Apicomplexans are obligative parasites whose life cycle employs both asexual and sexual forms of reproduction. Hematophagous invertebrates serve as definitive hosts and one of numerous species of vertebrates serve as intermediate hosts. Members of the orders Haemosporida, Piroplasmida, Eimeriida, and Adeleida infect bats.

Bats serve as intermediate hosts for several Haemosporidia genera. The wingless Nycteribiidae bat flies are the primary invertebrate vector for most groups of haemosporidians, however *Penicillidia* flies and *Anopheles* mosquitos also are vectors. The Haemosporidia genera reported in bats are follows: *Plasmodium, Polychromophilus, Hepatocystis, Bioccala, Nycteria, Johnsprentia, Sprattiellai*, and *Biguetiella*. Of the *Plasmodia* species, *P. voltaicum* parasites are found exclusively in two species of African frugivorous bats and *P. cyclopsi* is found in the insectivorous bat *H. cyclops*. The *Plasmodia* of bats appear to be most closely related to those of rodents. By contrast, *Anopheles* mosquitoes are the vectors for the *Plasmodia* species that cause malaria in humans. *Polychromophilus* species parasites are found in insectivorous *Miniopterus*

bats and are believed to be more closely related to avian, rather than mammalian, parasites. Unlike humans, infection of bats does not lead to anemia. A large number of different *Hepatocystis* parasites are highly prevalent in mainly frugivorous and nectivorous species of bats and reside primarily in Oceania, Asia, and Southeast Asia, with fewer numbers of bats living in Africa. They rarely cause disease. By contrast, two species of *Bioccala* have been reported in a variety of both Old and New World bats. They inhabit exclusively insectivorous bats found in temperate regions. The *Nycteria* group of parasites only infects bats of the Rhinolophidae or Nycteridae families. One member each of the *Johnsprentia* and *Sprattiella* species have been reported in *Pteropus alecto* fruit bats in Oceania and one species of *Biguetiella* is found in one species of insectivorous bat in Africa. Haemosporidians from bats thus do not infect humans and are more closely related to parasites of other mammals or birds.

The order Piroplasmida includes several genera with members that are highly pathogenic to humans or cattle. *Babesia vesperuginis* causes a malaria-like illness in several British bat species and one bat species from South America. *B. canis canis*, typically found in dogs, is also present in four European bats, while one species of *Myotis* contains the cattle pathogen *B. besnoiti* in its guano. While present in bats from many different regions of the world, *Babesia* species have only been reported in insectivorous bats, almost exclusively of the family Vespertilionoidea. A malaria-like illness in humans is caused by *Babesia microti or Babesia divergensi* (Beltz 2011). *Klossiella* species coccidians have been reported in the kidneys of several insectivorous North American and an African bat species. Several studies found unidentified coccidians in either dead insectivorous bats or bats with severe coccidiosis of the kidney tubules and on the kidney surface. Members of the Piroplasmidia order thus contain several severe pathogens of insectivorous bats, humans, and domestic animals.

Several genera of the order Eimeriida (*Toxoplasma* and *Eimeria*) may cause severe disease or death in immunocompromised humans and several domestic animals. Infection of pregnant women with *Toxoplasma gondii* causes neurological disease in the fetuses or miscarriage and may result in fatal infection in HIV-positive people. Cats are the only known definitive host, but many vertebrates serve as intermediate hosts. Infection with *T. gondii* leads to respiratory distress, neurological disease, and death in two species of Australian flying foxes. Many species of bats worldwide are either infected with *T. gondii* or produce anti-*T. gondii* IgG antibodies. Similarly, multiple species of *Eimeria* infect many bat species throughout the world. While *Eimeria tenella* causes severe disease in domestic fowl, this eimerian has not been reported in bats. Many bat eimerians belong to clades which include eimerians of rodents.

The Adeleida order includes members of the *Cryptosporidium* genus. *C. parvum* may be characterized as a group of closely related parasites composed of multiple genotypes, some of which may cause cryptosporidiosis, a potentially fatal disease in immunocompromised individuals. It also occasionally leads to gastrointestinal disease among immunocompetent people. In addition to humans, rodent genotypes exist. Several bat *Cryptosporidium* genotypes (bat genotypes I–VII) may be found in regions throughout most of the world, with the exception of Sub-Saharan Africa. Of these, bat genotype V is similar to the human cryptosporidiosis clade and thus might be able to infect humans. Insectivorous, frugivorous, and nectivorous bats may all serve as hosts for *Crytosporidium*.

Taken together, many members of the genus Apicomplexa are found in mammalian species throughout the world, including bats, rodents, and humans. Some cause severe

to life-threatening illness, especially in immunosuppressed people, while others cause minimal disease at most in humans. Bats are also susceptible to severe disease or death from some of these parasites. The present evidence suggests that bats are unlikely to serve as reservoir hosts for zoonotic disease in humans or domestic animals, with possible exceptions of *T. gondii* and bat *Cryptosporidium* genotype V. Defining the relevant intermediate hosts for Apicomplexans, particularly *T. gondii* and *Crytoporidium* species, as well as the major means of intra- or interspecies transmission may allow guidelines to be developed to reduce infection of at-risk groups of humans and animals, similar to current recommendations for pregnant women to avoid exposure to cat feces.

REFERENCES

Afonso E, Baurand P-E, Tournant P, Capelli N. 2014. First amplification of *Eimeria hessei* DNA from the lesser horseshoe bat (*Rhinolophus hipposideros*) and its phylogenetic relationships with *Eimeria* species from other bats and rodents. *Experimental Parasitology.* 139:58–62.

Alyousif MS, Al-Dakhil M, Al-Shawa Y. 1999. *Eimeria pipistrellus* n. sp. from *Pipistrellus kuhlii* (Chiroptera: Vespertilionidae) in Saudi Arabia. *The Korean Journal of Parasitology*. 37(1):1–4.

Beltz LA. 2011. *Foundations of Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey-Bass and APHA Press: San Francisco, CA.

Bouland, Y. 1975. Etude morphologique des coccidies (Adeleidae). *Klossiella killicki* n. sp. chez des microchiropterae Africans et *Klossiella tejerai Scorza* chez un marsupial sud-americain. *Bulletin of the Museum of Natural History*, 3rd series no. 284, *Zoology*. 194:83–89.

Bray RS. 1958. On the parasitic protozoa of Liberia. I. Coccidia of some small mammals. *Journal of Protozoology*. 5:81–83.

Breinl A. 1911. Report of the year 1911. Australian Institute of Tropical Medicine: Townsville.

Cabral AD, D'Auria SRN, Camargo MCGO, Rosa AR, Sodré MM, Galvão-Dias MA, Jordão LR, Dubey JP, Gennari SM, Pena HFJ. 2014. Seroepidemiology of *Toxoplasma gondii* infection in bats from São Paulo city, Brazil. *Veterinary Parasitology*. 206:293–296.

Cabral AD, Gama AR, Sodré MM, Savani ESMM, Galvão-Dias MA, Jordão LR, Maeda MM, Yai LEO, Gennari SM, Pena HFJ. 2013. First isolation and genotyping of *Toxoplasma gondii* from bats (Mammalia: Chiroptera). *Veterinary Parasitology.* 193:100–104.

Dionisi A. 1899. La malaria di alcune specie di pipistrelli. *Atti della Società per gli Studi della Malaria.* 1:133–175.

Dodd NS, Lord JS, Jehle R, Parker S, Parker F, Brooks DR, Hide G. 2014. *Toxoplasma gondii*: Prevalence in species and genotypes of British bats (*Pipistrellus pipistrellus* and *P. pygmaeus*). *Experimental Parasitology*. 139:6–11.

Dubey PA, Hamir N, Sonn AM, Topper MJ. 1998. Cryptosporidiosis in a bat (*Eptesicus fuscus*). *Journal of Parasitology.* 84:622–623.

Duszynski DW. 1997. Coccidia from bats (Chiroptera) of the world: a new *Eimeria* species in *Pipistrellus javanicus* from Japan. *Journal of Parasitology.* 83(2):280–282.

Duszynski DW, Barkley LJ. 1985. *Eimeria* from bats of the world: a new species in *Tomopeas ravus* from Peru. *Journal of Parasitology.* 71(2):204–208.

Duszynski DW, Reduker DW, Parker BB. 1988. *Eimeria* from bats of the world. II. A new species in *Tadarida femorosacca* from Sonora, Mexico. *Journal of Parasitology*. 74(2):317–321.

Duszynski DW, Scott DT, Aragon J, Leach A, Perry T. 1999a. Six new *Eimeria* species from vespertilionid bats of North America. *Journal of Parasitology.* 85(3):496–503.

Duszynski DW, Scott DT, Zhao X. 1999b. *Eimeria* from bats of Bolivia: two new species from vespertilionid bats. *Journal of Parasitology.* 85(3):504–507.

Duval L, Mejean C, Maganga GD, Makanga BK, Koumba LBM, Peirce MA, Ariey F, Bourgarel M. 2012. The chiropteran haemosporidian *Polychromophilus melanipherus*: A worldwide species complex restricted to the family Miniopteridae. *Infection, Genetics and Evolution.* 12:1558–1566.

Duval L, Robert V, Csorba G, Hassanin A, Randrianarivelojosia M, Walston J, Nhim T, Goodman SM, Ariey F. 2007. Multiple host-switching of Haemosporidia parasites in bats. *Malaria Journal.* 6:157.

Eyles DE, Dunn FL, Liat LB. 1962. Blood parasites of Malayan bats. *Medical Journal of Malaya.* 17:87–88.

Fournier GFdSR, Lopes MG, Marcili A, Ramire DG, Acosta ICL, Ferreira JIGdS, Cabral ADC, de Lima JTR, Pena HFdJ, Dias RA, Gennari SM. 2014. *Toxoplasma gondii* in domestic and wild animals from forest fragments of the municipality of Natal, northeastern Brazil. *Brazilian Journal of Veterinarian Parasitology.* 23(4):501–508.

Gardner RA, Molyneux DH. 1987. *Babesia vesperuginis*: natural and experimental infections in British bats (Microchiroptera). *Parasitology.* 95(3):461–469.

Gardner RA, Molyneux DH. 1988. *Polychromophilus murinus*: a malarial parasite of bats: life-history and ultrastructural studies. *Parasitology.* 96(3):591–605.

Garnham PCC. 1966. *Malaria parasites and other Haemosporidia*. Blackwell: Oxford, p. 1114.

Garnham PCC, Heisch RB. 1953. On a new blood parasite of insectivorous bats. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 47:357–363.

Garnham PCC, Lainson R, Shaw JJ. 1971. A contribution to the study of the haematozoon parasites of the bats. A new mammalian haemoproteid, *Polychromophilus deanei* n. sp. *Memórias do Instituto Oswaldo Cruz*. 69:119–125.

Gazzonis EL, Garcia GA, Zanzani SA, Garippa A, Rossi L, Maggiora M, Dini V, Invernizzi A, Luini M, Tranquillo VT, Mora LO, Manfredi MT. 2014. *Besnoitia besnoiti* among cattle in insular and northwestern Italy: endemic infection or isolated outbreaks? *Parasites & Vectors.* 7:585.

Gruber AD, Schulze CA, Brugmann M, Pohle J. 1996. Renal coccidiosis with cystic tubular dilatation in four bats. *Veterinarian Pathology.* 33:442–445.

Hornok S, Estók P, Kováts D, Flaisz B, Takács N, Szőke K, Krawczyk A, Kontschán J, Gyuranecz M, Fedák A, Farkas R, Haarsma A-J, Sprong H. 2015. Screening of bat faeces for arthropod-borne apicomplexan protozoa: *Babesia canis* and *Besnoitia besnoiti*-like sequences from Chiroptera. *Parasites & Vectors.* 8:441.

Jiang HH, Qin SY, Wang W, He B, Hu TS, Wu JM, Fan JS, Tu CC, Liu Q, Zhu XQ. 2014. Prevalence and genetic characterization of *Toxoplasma gondii* infection in bats in southern China. *Veterinary Parasitology.* 203:318–321.

Krampitz HE, Anciaux De Faveaux FM. 1960. Über einiger Haemosporidien aus Fledermaüsen der Höhlen des Berglandes Katanga. *Zeitschrift für Tropenmedizin und Parasitologie.* 11:391–400.

Kusewitt DF, Wagner JE, Harris PD. 1977. *Klossiella* sp. in the kidneys of two bats (*Myotis sodalis*). *Veterinary Pathology*. 3(4):365–369.

Kváč M, Hořická A, Sak B, Prediger J, Salát J, Širmarová J, Bartonička T, Clark M, Chelladurai JR, Gillam E, McEvoy J. 2015. Novel *Cryptosporidium* bat genotypes III and IV in bats from the USA and Czech Republic. *Parasitology Research.* 114(10):3917–3921.

Landau I. 1981. Description d'*Hepatocystis garnhami* n. sp., parasite du chiroptère *Cynopterus sphinx* (Vahl) en Thaïlande. In: *Parasitological Topics*. A presentation volume to P.C.C. Garnham, F.R.S., on the occasion of his 80th Birthday, 1981. EU Canning (ed.). Society of Protozoologists, Allen Press: Lawrence, KS, pp. 166–169.

Landau I, Adam JP. 1971. Description de schizontes de rechute chez un nouvel haemoproteidae, *Hepatocystis perronae* n. sp., parasite de Megachiroptères africains. *Cahier ORSTOM, Série Entomologie Médicale et Parasitologie*. 9:373–378.

Landau I, Baccam D, Ratanaworabhan N, Yenbutra S, Boulard Y, Chabaud AG. 1984. New Haemoproteidae parasites of Chiroptera in Thailand. *Annales de Parasitologie Humaine et Comparée.* 59(5):437–447.

Landau I, Chabaud AG. 1978. Description de *Plasmodium cyclopsi* n. sp. parasite du microchiroptère *Hipposideros cyclops* à Makokou (Gabon). *Annales de Parasitologie Humaine et Comparée*. 53:247–253.

Landau I, Chabaud AG, Miltgen F, Baccam D. 1980a. *Dionisia bunoi* n. gen., n. sp. Haemoproteidae parasite du microchiroptère *Hipposideros cyclops* au Gabon. *Annales de Parasitologie Humaine et Comparée*. 55:271–280.

Landau I, Chavatte JM, Beveridge I. 2012a. *Johnsprentia copemani* gen. nov., sp. nov. (Haemoproteidae), a parasite of the flying-fox, *Pteropus alecto* (Pteropodidae). *Memoirs of the Queensland Museum*. 5661–5666.

Landau I, Chavatte JM, Karadjian G, Chabaud A, Beveridge I. 2012b. The haemosporidian parasites of bats with description of *Sprattiella alecto* gen. nov., sp. nov. *Parasite.* 19:137–146.

Landau I, Humpherey-Smith I, Chabaud AG, Miltgen F, Copeman B, Boulard Y. 1985. Description et transmission expérimentale de l'haemoproteidé *Hepatocystis levinei* n.sp. *Annales de Parasitologie Humaine et Comparée*. 60:373–382.

Landau I, Miltgen F, Le Bail O, Yap LF. 1976. *Hepatocystis* de Malaisie IV. Description d'*Hepatocystis rodhaini* n. sp. parasite de Microchiroptères. *Annales de Parasitologie Humaine et Comparée*. 51:303–307.

Landau I, Rosin G, Miltgen F, Hugot JP, Leger N, Beveridge I, Baccam D. 1980b. Sur le genre *Polychromophilus* (Haemoproteidae, parasite de microchiroptères). *Annales de Parasitologie Humaine et Comparée.* 55:13–32.

Mandal AK, Nair KN. 1973. A new species of coccidium from *Taphozous melanopogon* Temminck (Mammalia: Chiroptera) from Andaman Islands. *Proceedings of the Indian Academy of Sciences*. 77B:243–246.

Manwell R, Kuntz RE. 1966. *Hepatocystis* of Formosan mammals with description of a new species. *Journal of Protozoology.* 13:670–672.

Marinkelle CJ. 1996. *Babesia* sp. in Columbian bats (Microchiroptera). *Journal of Wildlife Diseases*. 32(3):534–535.

Masbar S, Palmieri JR, Marwoto HA, Purnomo, Darwis F. 1981. Blood parasites of wild and domestic animals from South Kalimantan (Borneo), Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health.* 12(1):42–46.

McAllister CT, Burt S, Seville RS, Robison HW. 2011. A new species of *Eimeria* (Apicomplexa: Eimeriidae) from the eastern pipistrelle, *Perimyotis subflavus* (Chiroptera: Vespertilionidae), in Arkansas. *Journal of Parasitology.* 97(5):896–898.

McAllister CT, Seville RS, Arlen R, Connior MB. 2014. A new species of *Eimeria* (Apicomplexa: Eimeriidae) from tri-colored bats, *Perimyotis subflavus* (Chiroptera: Vespertilionidae), from the Ouachitas of Arkansas. *Acta Parasitology.* 59(4):690–693.

McAllister CT, Seville RS, Roehrs ZP. 2012. A new species of *Eimeria* (Apicomplexa: Eimeriidae) from the northern myotis, *Myotis septentrionalis* (Chiroptera: Vespertilionidae), in Oklahoma. *Journal of Parasitology.* 98(5):1003–1005.

McAllister CT, Upton SJ. 2009. Two new species of *Eimeria* (Apicomplexa: Eimeriidae) from eastern red bats, *Lasiurus borealis* (Chiroptera:Vespertilionidae), in Arkansas and North Carolina. *Journal of Parasitology.* 95(4): 991–993.

Megali A, Yannic G, Christe P. 2011. Disease in the dark: molecular characterization of *Polychromophilus murinus* in temperate zone bats revealed a worldwide distribution of this malaria-like disease. *Molecular Ecology.* 20:1039–1048.

Mialhe E, Landau I. 1977. Description d'*Hepatocystis bainae* n. sp. (Haemoproteidae) parasite d'*Hipposideros galeritus*, Microchiroptère de Malaisie. *Annales de Parasitologie Humaine et Comparée*. 52:385–390.

Miltgen F, Landau I, Bradbury J. 1980. *Hepatocystis* d'*Hypsignathus monstrosus* (Pteropinae) au Gabon. Description d'*Hepatocystis carpenteri* n. sp. *Annales de Parasitologie Humaine et Comparée*. 55:485–490.

Miltgen F, Landau I, Rosin G, Erard C. 1977. *Hepatocystis brosseti* n. sp., Haemoproteidae parasite d'*Epomops franqueti*, Pteropinae, au Gabon. *Annales de Parasitologie Humaine et Comparée*. 52:589–596.

Morgan UM, Sturdee AP, Singleton G, Gomez MS, Gracenea M, Torres J, Hamilton SG, Woodside SP, Thompson RCA. 1999. The *Cryptosporidium* "mouse" genotype is conserved across geographic areas. *Journal of Clinical Microbiology.* 37(5):1302–1305.

Morsy TA, Khaled ML, Bebars MA, Ramadan NF, Abdel Hamid MY. 1987. *Nycteria medusiformis* Garnham and Heisch, 1953: a malaria parasite of the Egyptian insectivorous bat, *Taphozous perforatus*. *Journal of Egyptian Society of Parasitology.* 17(1):1–7.

Murakoshi F, Recuenco FC, Omatsu T, Sano K, Taniguchi S, Masangkay JS, Alviola P, Eres E, Cosico E, Alvarez J, Une Y, Kyuwa S, Sugiura Y, Kato K. 2016. Detection and molecular characterization of *Cryptosporidium* and *Eimeria* species in Philippine bats. *Parasitology Research.* 115(5):1863–1869.

Paludo GR, Friedmann H, Dell'Porto A, Macintire DK, Whitley EM, Boudreaux MK, Baneth G, Blagburn BL, Dykstra CC. 2005. *Hepatozoon* spp.: pathological and partial 18S rRNA sequence analysis from three Brazilian dogs. *Parasitology Research*. 97(2):167–170.

Pinto CM, Helgenjj KM, Fleischer RC, Perkins SL. 2013. *Hepatozoon* parasites (Apicomplexa: Adeleorina) in bats. *Journal of Parasitology.* 99(4):722–724.

Qin SY, Cong W, Liu Y, Li N, Wang Z-D, Zhang F-K, Huang S-Y, Zhu X-Q, Liu Q. 2014. Molecular detection and genotypic characterization of *Toxoplasma gondii* infection in bats in four provinces of China. *Parasites & Vectors.* 7:558.

Roberts LS, Janovy J Jr. 2006. *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology*. McGraw-Hill: New York, NY.

Rodhain J. 1926. *Plasmodium epomophori* n. sp., parasite commundes roussettes épaulières au Congo Belge. *Bulletin de la Société de Pathologie Exotique*. 8:726–729.

Rosin G, Landau I, Hugot JP. 1978. Considérations sur le genre *Nycteria* (Haemoproteidae) parasite de Microchiroptères africains avec description de 4 espèces nouvelles. *Annales de Parasitologie Humaine et Comparée.* 53:447–459.

Sangster CR, Gordon AN, Hayes D. 2012. Systemic toxoplasmosis in captive flying-foxes. *Australian Veterinary Journal.* 90(4):140–142.

Schaer J, Perkins SL, Decher J, Leendertz FH, Fahr J, Weber N, Matuschewski K. 2013. High diversity of West African bat malaria parasites and a tight link with rodent *Plasmodium* taxa. *Proceedings of the National Academy of Sciences.* 110(43):17415–17419.

Schaer J, Reeder DM, Vodzak ME, Olival KJ, Weber N, Mayer F, Matuschewski K, Perkins SL. 2015. *Nycteria* parasites of Afrotropical insectivorous bats. *International Journal for Parasitology.* 45:375–384.

Scott DT, Duszynski DW. 1997. *Eimeria* from bats of the world: two new species from *Myotis* spp. (Chiroptera: Vespertilionidae). *Journal of Parasitology*. 83(3):495–501.

Seville RS, Gruver J. 2004. Species of *Eimeria* (Apicomplexa: Eimeriidae) from bats Chiroptera:Vespertilionidae) in central Wyoming. *Journal of Parasitology.* 90(2):348–351.

Sun H, Wang Y, Zhang Y, Ge W, Zhang F, He B, Li Z, Fan Q, Wang W, Tu C, Li J, Liu Q. 2013. Prevalence and genetic characterization of *Toxoplasma gondii* in bats in Myanmar. *Applied and Environmental Microbiology.* 79(11):3526–3528.

Van der Kaay HJ. 1964. Description of a new *Plasmodium*: *Plasmodium voltaicum* sp. nov. found in a fruit bat, *Roussetus smithi* in Ghana. *Annals of Tropical Medicine and Parasitology*. 58:261–264.

Van Riel J, Herniaux-L'hoest D, Herniaux-L'hoest J. 1951. Description of a *Plasmodium* found in bat, *Roussetus leachi*. *Parasitology.* 41:270–273.

Wang W, Cao L, He B, Li J, Hu T, Zhang F, Fan Q, Tu C, Liu Q. 2013. Molecular characterization of *Cryptosporidium* in bats from Yunnan Province, Southwestern China. *Journal of Parasitology.* 99(6):1148–1150.

Wheat BE. 1975. *Eimeria Macyi* (*sic*) sp. n. (Protozoa: Eimeriidae) from the eastern pipistrelle, *Pipistrellus subflavus*, from Alabama. *Journal of Parasitology*. 61(5):920–922.

Witsenburg F, Schneider F, Christe P. 2014. Epidemiological traits of the malaria-like parasite *Polychromophilus murinus* in the Daubenton's bat *Myotis daubentonii*. *Parasites & Vectors.* 7:566.

Wünschmann A, Wellehan JFX Jr, Armien A, Bemrick WJ, Barnes D, Averbeck GA, Roback R, Schwabenlander M, D'Almeida E, Joki R, Childress AL, Cortinas R, Gardiner CH, Greiner EC. 2010. Renal infection by a new coccidian genus in big brown bats (*Eptesicus fuscus*). *Journal of Parasitology.* 96(1):178–183.

Yuan Z-G, Luo SJ, Dubey JP, Zhou D-H, Zhu Y, He Y, He X-H, Zhang XX, Zhu X-Q. 2013. Serological evidence of *Toxoplasma gondii* infection in five species of bats in China. *Vector-Borne and Zoonotic Diseases.* 13(6):422–424.

Zhang XX, Huang SY, Zhang YG, Zhang Y, Zhu XQ, Liu Q. 2014. First report of genotyping of *Toxoplasma gondii* in free-living *Microtus fortis* in Northeastern China. *Journal of Parasitology.* 100:692–694.

Zhao X, Duszynski DW, Loker ES. 2001. Phylogenetic position of *Eimeria antrozoi*, a bat coccidium (Apicomplexa: Eimeriidae) and its relationship to morphologically similar *Eimeria* spp. from bats and rodents based on nuclear 18 s and plastid 23 s rDNA sequences. *Journal of Parasitology*. 87(5):1120–1123.

12

KINETOPLASTIDS AND BATS

12.1 KINETOPLASTIDS

Kinetoplastids are protozoan blood parasites found throughout the world. They parasitize almost all orders of vertebrates and invertebrates. Trypanosomatids, including *Trypanosoma* and *Leishmania* species, have the second widest range of hosts and geographical distribution among eukaryotes (Hoare 1972). Their life cycle alternates between invertebrate hosts (typically hematophagous arthropods) and vertebrates (Acosta *et al*. 2014). Transmission is via fecal or salivary material of leeches and bloodsucking arthropods. Morphologically, the bloodstream trypomastigote form of *Trypanosoma* may be used to divide trypanosome species into those with large, broad trypomastigotes of the subgenus *Megatrypanum* and those with small, slender forms of the subgenus *Schizotrypanum*. Approximately 70 species of bats throughout the world are infected by members of *Schizotrypanum* (Baker *et al*. 1978). See Table 12.1 for a list of various kinetoplastids with reported association to bats.

Kinetoplastids contain a unique structure, the kinetoplast, located near the basal body at the base of their flagella. This structure is part of the mitochondria and contains DNA consisting of mini-circles and maxi-circles. A flagellum is present at some stage of the kinetoplastid life cycle. This may be attached to part of the cell membrane to form an undulating membrane used for mobility.

The life cycle of kinetoplastids includes several different morphological forms which are found in different host species. In the trypomastigote form, the kinetoplast is

Bats and Human Health: Ebola, SARS, Rabies and Beyond, First Edition. Lisa A. Beltz.

Companion website: www.wiley.com/go/batsandhumanhealth

TABLE 12.1 Kinetoplastids associated with bats

Bat family	Bat common name	Bat species	Parasite
Phyllostomidae	Geoffroy's tailless bat	*Anoura geoffroyi*	*Trypanosoma cruzi* clade
Vespertilionidae	Pallid bat	*Antrozous pallidus*	*Trypanosoma dionisii*
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	*Leishmania mexicana*
Phyllostomidae	Jamaican fruit-eating bat	*Artibeus jamaicensis*	*Trypanosoma cruzi* clade
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Leishmania mexicana*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Trypanosoma cruzi* clade
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Flat-faced fruit-eating bat	*Artibeus planirostris*	*Trypanosoma rangeli*
Rhinolophoidea	Heart-nosed bat	*Cardioderma cor*	*Leishmania tropica*
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Leishmania infantum chagasi*
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Trypanosoma dionisii*
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Trypanosoma cruzi* – TcI
Phyllostomidae	Seba's short-tailed bat	*Carollia perspicillata*	*Trypanosoma cruzi* – TcBat
Phyllostomidae	Sowell's short-tailed bat	*Carollia sowelli*	*Leishmania mexicana*
Vespertilionidae	Gould's wattled bat	*Chalinolobus gouldii*	*Trypanosoma vegrandis*
Phyllostomidae	Godman's long-tailed bat	*Choeroniscus godmani*	*Leishmania mexicana*
Phyllostomidae	Big-eared wooly bat	*Chrotopterus auritus*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Pygmy fruit-eating bat	*Dermanura phaeotis*	*Leishmania mexicana*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Leishmania amazonensis*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Leishmania infantum*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Leishmania mexicana*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Trypanosoma cruzi marinkellei*
Vespertilionidae	Brazilian brown bat	*Eptesicus brasiliensis*	*Trypanosoma dionisi*
Vespertilionidae	Serotine bat	*Eptesicus serotinus*	*Trypanosoma dionisi*
Phyllostomidae	Commissaris's long-tongued bat	*Glossophaga commissarisi*	*Leishmania mexicana*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Leishmania mexicana*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Trypanosoma cruzi* clade
Rhinolophidae	Dusky horseshoe bat	*Hipposideros ater*	*Trypanosoma hipposideri*
Rhinolophidae	Sundevall's roundleaf bat	*Hipposideros caffer*	*Trypanosoma livingstonei*
Phyllostomidae	Lesser short-nosed bat	*Leptonycteris curasoae*	*Leishmania mexicana*
Phyllostomidae	White-throated round-eared bat	*Lophostoma silvicolum*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Fruit bats	*Micronycteris* sp.	*Trypanosoma cruzi marinkellei*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Leishmania* sp.

Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Trypanosoma dionisii*
Molossidae	Angolan free-tailed bat	*Mopys condylurus*	*Trypanosoma erneyi*
Vespertilionidae	Silver-tipped myotis	*Myotis albescens*	*Trypanosoma cruzi*
Vespertilionidae	Brandt's bat	*Myotis brandtii*	*Trypanosoma dionisii*
Vespertilionidae	Yellowish myotis	*Myotis levis*	*Trypanosoma cruzi* – Tcbat
Vespertilionidae	Black bat	*Myotis nigricans*	*Trypanosoma cruzi marinkellei*
Vespertilionidae	Red myotis	*Myotis ruber*	*Trypanosoma cruzi*
Noctionidae	Lesser bulldog bat	*Noctilio albiventris*	*Trypanosoma cruzi*
Noctionidae	Southern bulldog bat	*Noctilio labialis*	*Trypanosoma cruzi* – TcBat
Vespertilionidae	Lesser noctule	*Nyctalus leisleri*	*Trypanosoma dionisii*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Trypanosoma dionisii*
Vespertilionidae	Common noctule	*Nyctalus noctula*	*Trypanosoma vespertilionis*
Emballonuroidea	Hairy slit-faced bat	*Nycteris hispida*	*Leishmania major*
Vespertilionidae	Evening bat	*Nycticeius humeralis*	*Blastocrithidia*
Vespertilionidae	Evening bat	*Nycticeius humeralis*	*Trypanosoma cruzi* – TcI
Vespertilionidae	Lesser long-eared bat	*Nyctophilus geoffroyi*	*Trypanosoma vespertilionis*
Vespertilionidae	Western pipistrelle	*Parastrellus hesperus*	*Trypanosoma dionisii*
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	*Leishmania mexicana*
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	*Trypanosoma cruzi marinkellei* II
Phyllostomidae	Pale spear-nosed bat	*Phyllostomus discolor*	*Trypanosoma cruzi marinkellei* I
Phyllostomidae	Lesser spear-nosed bat	*Phyllostomus elongates*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	*Trypanosoma cruzi marinkellei* I
Vespertilionidae	Common pipistrelle	*Pipistrellus pipistrellus*	*Trypanosoma vespertilionis*
Vespertilionidae	Common pipestrelle	*Pipistrellus pipistrellus*	*Trypanosoma dionisii*
Phyllostomidae	White-lined broad-nosed bat	*Platyrrhinus lineatus*	*Trypanosoma rangeli*
Molossidae	Mastiff bat	*Promops nasutus*	*Trypanosoma dionisii*
Mormoopidae	Big naked-back bat	*Pteronotus gymnonotus*	*Trypanosoma wauwau*
Mormoopidae	Parnell's mustached bat	*Pteronotus parnilli*	*Trypanosoma cruzi* – TcI
Mormoopidae	Parnell's mustached bat	*Pteronotus parnilli*	*Trypanosoma cruzi* – TcBat
Mormoopidae	Parnell's mustached bat	*Pteronotus parnilli*	*Trypanosoma cruzi marinkellei*
Mormoopidae	Parnell's mustached bat	*Pteronotus parnilli*	*Trypanosoma wauwau*
Mormoopidae	Wagner's mustached bat	*Pteronotus personatus*	*Leishmania mexicana*
Pterpodidae	Black flying fox	*Pteropus alecto*	*Trypanosoma pteropid*
Pterpodidae	Little red flying fox	*Pteropus scapulatus*	*Trypanosoma teixeirae*
Pterpodidae	Little red flying fox	*Pteropus scapulatus*	*Trypanosoma vegrandis*

(*Continued*)

TABLE 12.1 (Continued)

Bat family	Bat common name	Bat species	Parasite
Pterpodidae	White-lined broad-nosed bat	*Platyrrhinus lineatus*	*Leishmania* species
Rhinolophidae	Lander's horseshoe bat	*Rhinolophus landeri*	*Trypanosoma livingstonei*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lillium*	*Leishmania mexicana*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lillium*	*Trypanosoma dionisii*
Phyllostomidae	Highland yellow-shouldered bat	*Sturnira ludovici*	*Leishmania mexicana*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Blastocrithidia*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Trypanosoma dionisii*
Molossidae	Free-tailed bats	*Tadarida* sp.	*Trypanosoma erneyi*
Emballonuridae	Naked-rumped tomb bat	*Taphozous nudiventris*	*Trypanosoma longiflagellum*
Thyropteridae	Spix's disk-winged bat	*Thyroptera tricolor*	*Trypanosoma cruzi* – TcI
Phyllostomidae	Greater round-eared bat	*Tonatia bidens*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Fringed-lipped bat	*Trachops cirrhosis*	*Trypanosoma cruzi marinkellei*
Phyllostomidae	Great-stripe-nosed bat	*Vampyrodes caraccioli*	*Trypanosoma cruzi* –TcI

located at the parasite's posterior end. It contains a long undulating membrane. In the epimastigote form, the kinetoplast is centrally located, anterior to the nucleus. The undulating membrane is relatively short. In the promastigote form, the kinetoplast is at the anterior end and it contains no undulating membrane. The amastigote form is more spherical and lacks a free flagellum (Tulane University 2016).

12.2 TRYPANOSOMES

Trypanosomes differ in their life cycles, using different hosts, different vectors using different routes of host inoculation, and different morphological forms. They also are cause very different diseases, which may or may not be curable.

12.2.1 Life cycles of trypanosomes

12.2.1.1 The life cycle of the Trypanosoma cruzi *group of kinetoplastids*

Transmission of the *T. cruzi* group of kinetoplastids to humans or other vertebrate hosts begins with a blood meal by an infected triatomine insect vector ("kissing bugs"). Metacyclic trypomastigotes present in the insect's feces are rubbed into the wound site. The trypomastigotes then invade host cells, including macrophages, and differentiate into intracellular amastigotes. The amastigotes multiply by binary fission, and then differentiate into bloodstream trypomastigotes, which are released into the circulatory system. These trypomastigotes infect a variety of cells and transform back into the replicating intracellular amastigote form. This process is repeated multiple times. The insect vector becomes infected by feeding on animal blood containing trypomastigotes. The trypomastigotes transform into epimastigotes in the vector's midgut and multiply there, eventually differentiating in the midgut into the metacyclic trypomastigotes in the hindgut. These are then transmitted to next vertebrate host to continue the life cycle (CDC 2016a).

12.2.1.2 The lifecycle of the Trypanosoma brucei *group of kinetoplastids*

Transmission of *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* to humans begins as the tsetse fly vector takes a blood meal and injects the parasites from their saliva into the host's skin. They enter the circulatory system, where they transform into bloodstream trypomastigotes and begin multiple rounds of replication extracellularly. The trypomastigote progeny are disseminated throughout the body, invading blood fluids, including lymph and spinal fluid. The tsetse fly vector is infected by bloodstream trypomastigotes during the course of a blood meal. In the fly's midgut, the bloodstream trypomastigotes transform into procyclic trypomastigotes which multiply by binary fission. Procyclic trypomastigotes then leave the midgut and transform into epimastigotes which enter the fly's salivary glands. From there, the parasites are transmitted to mammals during the fly's next blood meal. While humans serve as the primary reservoir for *T. b. gambiense*, it may also infect other animals. Wild game animals are the primary reservoirs of *T. b. rhodesiense* (CDC 2016b). *Trypanosoma brucei brucei* causes a fatal disease in cattle, harming the economy of the locale.

12.2.2 Trypanosomes and disease

12.2.2.1 **Trypanosoma cruzi *and Chagas disease*** *Trypanosoma cruzi cruzi* causes Chagas disease in humans. This may either be manifested as a mild, lifelong infection or may be fatal. The disease has an acute and a chronic phase. If untreated, infection is life-long. The early, acute phase of infection is typically mild or asymptomatic, although it can, in rare cases, lead to severe inflammation of the heart or brain and meninges. Most of those infected progress into the prolonged, asymptomatic, chronic stage of disease. Approximately 20–30% of infected people, however, develop a debilitating and life-threatening form of infection. This form is associated with potentially fatal heart arrhythmia, severe dilation of the heart that decreases its ability to pump blood, or dilation of the esophagus or colon which leads to difficulties with eating or passing stool (CDC 2016a).

12.2.2.2 **Trypanosoma brucei *and African sleeping sickness*** *T. b. gambiense* and *T. b. rhodesiense* are the causative agents of African sleeping sickness. This disease constitutes a serious public health problem in parts of Sub-Saharan Africa, with approximately 10 000 new cases reported each year. The parasites cross the blood–brain barrier into the central nervous system, where severe damage occurs, leading to death rapidly or over the course of several years (*T. b.gambiense* or *T. b. rhodesiense*, respectively). Sleeping sickness is curable with medication (CDC 2016b).

12.2.3 Trypanosomes infecting bats throughout the world

More than 30 trypanosome species have been reported in over 100 bat species throughout Africa, Asia, South America, Europe, and Oceania. Most of the infected bats are insectivorous and are exposed to trypanosomes by ingesting infected insects. Since bats are generally long-lived, infection may last for years, with trypanosomes residing in skeletal, cardiac, and stomach muscles (Garcia *et al*. 2012). The majority of trypanosomes in bats belong to the *Schizotrypanum* subgenus (Lima *et al*. 2013). *Schizotrypanum* species typically restrict their mammalian hosts to bats, with the exception of the generalist, *T. c. cruzi*. *Schizotrypanum* trypanosomes of bats include *Trypanosoma vegrandi, Trypanosoma hastatus*, and *Trypanosoma erneyi* in African bats; *Trypanosoma livingsoni* in Mozambique; *T. cruzi, Trypanosoma vespertilionis*, and *Trypanosoma pipistrelli* in European bats; *Trypanosoma pteropi* and *Trypanosoma hipposideri* in Australia; *Trypanosoma hedricki* and *Trypanosoma myotis* in North and Central America; and *Trypanosoma phyllostomae, Trypanosoma cruzi marinkellei*, and *Trypanosoma desterrensis* in Central and South America (Steindel *et al*. 1998; Grisard *et al*. 2003; Lima *et al*. 2012, 2013; Austen *et al*. 2015). *Trypanosoma dionisii* is found in bats from both Europe and South America. Its colonization of South America bats appears to be a relatively recent event (Cottontail *et al*. 2014) and new species of trypanosomes continue to be discovered and classified at a molecular level, rather than depending upon morphology alone.

Several trypanosome species found in bats are from the *Herpetosoma* subgenus. These include *Trypanosoma rangeli* in Brazilian bats, *Trypanosoma lewisi* in Puerto Rico, *Trypanosoma lineatum* in Venezuela, *Trypanosoma longiflagellum* in the naked-rumped tomb bat (*Taphozous nudiventris*) in Iraq, and *Trypanosoma aunauwa* in New Guinea. *T. rangeli* infects humans and domestic and sylvatic mammals in Central to

northwestern South America. Despite the presence of *T. rangeli* in bats and in its insect vector, *Rhodnius stali*, only a handful of humans are known to have been infected by this trypanosome in the Amazon region (Marinkelle 1977; da Silva *et al*. 2009).

12.2.3.1 Trypanosomes and bats in the Americas Bat hosts shown to be infected with trypanosomes include the following: *Pipistrellus pipistrellus* and *Nyctalus noctula* (infected by *T. vespertilionis*); *Platyrrhinus lineatus* and *Artibeus planirostris (T. rangeli); P. pipistrellus, N. noctula, Eptesicus brasiliensis, Sturnira lillium, Molossus molossus, Promops* species, and *Carollia perspicillata (T. dionisii); Tadarida* species and *Mopys condylurus (T. erneyi); Phyllostomus discolor, Phyllostomus hastatus, A. planirostris, C. perspicillata, Chrotopterus auritus, Desmodus rotundus, Lophostoma silvicolum, Tonatia bidens*, and *Trachops cirrhosus (T. c. marinkellei); C. perspicillata* and *Thyroptera* species (*T. cruzi* - TcI); and *Myotis levis* (*T. cruzi* - Tcbat). *T. cruzi* is also present in *Myotis nigricans, Myotis albescens, Myotis ruber, Noctilio albiventris*, and *Thyroptera tricolor* (Cavazzana Jr *et al*. 2010; Lima *et al*. 2013). In Bolivia, the prevalence of *T. c. marinkellei* in the bat family *Phyllostomidae* is 9.0% and the prevalence of *T. dionisii* is 7.0% (Garcia *et al*. 2012). *T. cruzi marinkellei* can be divided into two major subdivisions: *T. c. marinkellei* I in *P. discolor* and *P. hastatus* bats; and *T. c. marinkellei* II in *P. discolor* (Barnabe *et al*. 2003).

In Brazil, trypanosome-infected bats have been found in all biomes and in domestic (including bats in thatched roofs of human dwellings) or sylvatic settings (Lima *et al*. 2015a). *T. cruzi*-like trypanosomes were found in 23.1% of *Anoura geoffroyii* ($n=13$), 33.3% of *Artibeus jamaisensis* ($n=9$), 20.0% of *C. perspicillata* ($n=20$), 33.1% of *D. rotundus* ($n=1$), 66.7% of *P. discolor* ($n=24$), 32.6% of *P. hastatus* ($n=46$), 16.7% of *P. parnellii rubiginosa* ($n=6$), and 50.0% of *Trachops cirrhosis* ($n=2$) (Pinto & Bento 1986). A more recent study found that 32.4% of the isolates from Brazilian bats are *T. dionisii*, which infects 12 species of bats from four families in all of the studied biomes. About half of the isolates were *T. c. marinkellei*, which is typically restricted to phyllostomid bats, but has also been reported in Vespertilionidae bats (*M. nigricans*) (Acosta *et al*. 2014). The remaining isolates were of the human pathogen *T. c. cruzi*, found in both vespertilionid and phyllostomid bats (Cavazzana *et al*. 2010).

A novel species from the *T. cruzi* clade, *Trypanosoma wauwau*, is present in *Pteronotus parnellii, Pteronotus gymnonotus*, and *Pteronotus personatus* bats (Mormoopidae family) in the Brazilian Amazon (Lima *et al*. 2015b). *T. wauwau* is in a sister clade present in *Artibeus jamaicensis, Artibeus lituratus*, and *T. cirrhosus* phyllostomid bats as well as a clade of trypanosomes reported in indigenous marsupials and rodents of Australia. Prevalence of *T. wauwau* in *Pteronotus* bats was 26.5%; however, this trypanosome was not found in sympatric bat species or those sharing shelters with *Pteronotus*. This trypanosome is not infective to the triatomine vectors of *T. rangeli* or *T. cruzi*. A 2016 study of trypanosomes in bats conducted in the region of a hydroelectric project in the Brazilian Amazon rainforest found an overall prevalence of 5.6% ($n=157$) (da Costa *et al*. 2016). TcI was isolated from *Vampyrodes caraccioli*, while TcBat was present in *C. perspicillata. T. wauwau* was also found in *Pteronotus parnellii*. Construction of hydroelectric dams impacts the local environment in several ways, including removal of vegetation from the terrestrial environment and the formation of the lakes. This leads to loss of sylvatic habitats and combines with the presence of towns in the area to support dam workers to bring bats and their microbes into closer contact with humans (da Costa *et al*. 2016).

The resulting stress may lower the bats' immunity and could lead to disease among the bats and potential reservoir species whose habitats were disrupted.

A. jamaicensis from the Panama Canal Zone, important seed dispersers, were detected by PCR to be infected by at least five species of trypanosomes, all from the *T. cruzi* clade, suggesting that at least five independent colonization events brought trypanosomes into the New World (Cottontail *et al*. 2014). Pinto *et al*. (2012), however, reported that in Panama, Tcbat is the only trypanosome to infect *Artibeus* and that infection is common (11.6% prevalence). Several important triatomine insect vectors are present in the area*: Panstrongylus geniculatus, Panstrongylus rufotuberculatus, Rhodnius pallescens*, and *Microtriatoma trinidadensis*. These vectors were found in association with bats.

In Columbia, infected bats were primarily *P. hastatus* (50% of this bat species were infected), *G. soricina* (15%), *A. lituratus* (12%), *M. molossus* (12%), *C. perspicillata* (11%), and the blood-feeding *D. rotundus* (8%) (Thomas *et al*. 2007). A separate study found the incidence of the infected triatomine insect vectors in infected Columbian bats to be as follows*: T. c. cruzi* (51% of tested insects infected), *T. c. marinkellei* (9%), *T. dionisii* (13%), *T. rangeli* (21%), *T. evansi* (4%), and *T. theileri* (2%). Among the various *T. cruzi* groups, TcI comprised 60% of the isolates, TcII comprised 15%, TcIII 7%, TcIV 7%, and TcBAT 11% (Ramírez *et al*. 2014). Many of the infected bat species typically live on palms which are highly infested by the insect vector *R. prolixus*, shown to be naturally infected with *T. c. cruzi* and *T. rangeli* (Ramírez *et al*. 2014).

In Texas, in the southwestern portion of the US, a 2016 study tested bat blood and heart tissue for the presence of trypanosomes ($n=593$). The prevalence of *T. cruzi* TcI was 0.17%, and the prevalence for *T. dionisii* was 1.5%. Surprisingly, 0.8% of the Texas bats were positive for five new *Blastocrithidia* species, a group of trypanosomes that had previously only been found in insects. These novel *Blastocrithidia* were most closely matched to *Blastocrithidia triatomae* from a *Triatoma protracta* insect (Hodo *et al*. 2016). Both TcI and *Blastocrithidia* were present in *Nycticeius humeralis* bats (prevalence of 1.4% for both, $n=70$). *Tadarida brasiliensis* were positive for *T. dionisii* (prevalence of 1.1%) and *Blastocrithidia* (prevalence of 0.8%) ($n=476$ for both). *Parastrellus hesperus* were positive for *T. dionisii* (prevalence of 13.3%, $n=15$). *Antrozous pallidus* were also positive for *T. dionisii* (prevalence of 22.2%, $n=9$) (Hodo *et al*. 2016). Seven species of triatomine bugs reside in Texas and blood of a *N. humeralis* bat was reported in one of them, a *Triatoma gerstaeckeri* (Gorchakov *et al*. 2016). The two most common triatomines found in Texas are *T. gerstaeckeri* and *Triatoma sanguisuga*, 50–70% of which are infected by *T. cruzi* (Kjos *et al*. 2009; Curtis-Robles *et al*. 2015). A number of other wildlife species in Texas are also infected by *T. cruzi* and could possibly serve as reservoir hosts. These include striped skunks, raccoons, bobcats, coyotes, gray foxes, woodrats, and other rodents (reviewed by Hodo *et al*. 2016). Notably, the prevalence of infection in these other animals ranges from 14 to 75%, far greater than the 1.4% prevalence in Texas bats, suggesting that bats may play only a very minimal role as a *T. cruzi* reservoir in this area.

12.2.3.2 Trypanosomes in Australia In Australia, several trypanosome species infect flying foxes. *T. pteropi* infects the black flying fox (*Pteropus alecto*), *T. hipposideri* infects the dusky horseshoe bat (*Hipposideros ater*), and a novel un-named trypanosome was found in an adult little red flying fox (*Pteropus scapulatus*). *T. vegrandis* is also

common in *Pteropus scapulatus, Nyctophilus geoffroyi*, and *Chalinolobus gouldii* and appears to be restricted to Australia, with an infection rate exceeding 80%. This trypanosome is geographically dispersed throughout Australia and has a low level of host specificity, infecting kangaroos, wallabies, bandicoots, and koalas (Thompson *et al*. 2014; Austen *et al*. 2015). It should be noted that all of the flying foxes tested in the latter report were clinically infected with Australian bat lyssavirus and obtained from a wildlife care facility. The results from this study may therefore differ from results involving healthy, wild bats.

A 2016 report detected a novel trypanosome species of the *T. cruzi* clade in an adult female little red flying fox (*Pteropus scapulatus*). Both the trypomastigote and round epimastigote form were seen in the blood films. Interestingly, the bat was moribund and displayed symptoms consistent with trypanosomiasis, such as anemia and jaundice. Utilizing both morphologic and molecular means, the trypanosome was determined to be a distinct species with a proposed name of *Trypanosoma teixeirae* (Barbosa *et al*. 2016).

12.2.3.3 Trypanosomes in Britain A study of bats in Britain isolated trypanosomes, primarily *T. dionisii*, from *Pipistrellus pipistrellus, Nyctalus leisleri, N. noctula, Eptesicus serotinus*, and *Myotis brandti. Cimex pipistrelli* bat bugs collected from bat roosts were also infected (60% prevalence, $n=20$), indicating that these bat bugs are vectors for trypanosomes in British bats (Gardner & Molyneux 1988).

12.2.4 *Trypanosoma cruzi*

T. c. cruzi is present in enzootic cycles in parts of the southern US and Mexico to southern South America. It is a generalist, infecting species of almost all mammalian orders, including domestic animals, such as dogs and chickens. In its sylvatic cycle, various groups of *T. cruzi* infect opossums, armadillos, raccoons, and wild nonhuman primates (Marcili *et al*. 2009). *T. cruzi* infects bats in several sylvatic niches and also those roosting in man-made structures, including buildings and dwelling lofts. Their presence not only attracts the triatomine insect vectors from nearby ecotopes but their infected blood serves as a source of parasites for previously uninfected triatomes. Bats may acquire parasites via contact with infected insect feces, ingestion of infected bugs, or congenitally (Marcili *et al*. 2009). Bat grooming habits may also transmit trypanosomes to bats via cimicid ectoparasites (Lima *et al*. 2013). Identification of trypanosome species in bats is complicated by their great morphological similarity and by the presence of mixed infection with several trypanosome groups, including species restricted to bats. Additionally, *T. cruzi* isolates demonstrate a high degree of heterogeneity. The genetic distances among *T. cruzi* isolates obtained from *P. hastatus* dwelling in the same hollow buriti palm tree averaged 0.35. This amount of heterogeneity suggests that these bats acquired the parasites from different infection sources. *P. hastatus* are avid insect feeders and the oral route is a common mechanism by which some groups of mammals become infected with *T. cruzi* (Lisboa *et al*. 2008).

Ecological parameters influence infections present in bats. In blood samples from 257 common fruit bats (*A. jamaicensis*) from a tropical lowland forest in Panama, 6.6% were found to be infected with variants of the *T. cruzi* complex of parasites. Trypanosome prevalence was greater in bats from areas with fragmented forest than in bats from regions of continuous forest (Cottontail *et al*. 2009).

According to the "bat-seeding hypothesis", *T. cruzi* originated as a bat trypanosome that expanded its host range into New World terrestrial mammals that modified its transmission to triatomine bugs (Hamilton *et al*. 2012; Lima *et al*. 2015a). The *T. cruzi* clade contains all sampled bat trypanosomes whether originating from Africa, Europe, or South America. This clade is postulated to have derived from an ancestral group of trypanosomes restricted to, and evolving exclusively in, bats after multiple spill-over events into terrestrial mammals, rather than a terrestrial clade that jumped into bats (Hamilton *et al*. 2012).

T. cruzi may be divided into two subspecies that are primarily found in bats: *T. c. cruzi* and *T. c. marinkellei* (found only in the Americas) (Baker *et al*. 1978). *T. c. cruzi* is further subdivided into seven discrete typing units classified as TcbI–TcbVI and *Trypanosoma Tcbat* (Tcbat), the latter found only in South and Central American bats. The trypanosomes' complex transmission cycle involves networks that may involve humans and other terrestrial mammals in addition to triatomine bugs that serve as the parasite's reservoir and vector.

12.2.4.1 Tcbat Tcbat from Brazilian bats have unique patterns of ribosomal and spliced leader PCRs. Phylogenic studies using SSUrRNA (small subunit of ribosomal rRNA), gGAPDH (glycosomal glyceraldehyde 3-phosphate dehydrogenase), Cytb (cytochrome b), or histone H2B genes suggest that Tcbat is a monophyletic lineage that is predominant in Brazil, Panama, and Colombia (Marcili *et al*. 2009; Pinto *et al*. 2012, 2015; Lima *et al*. 2015a). Tcbat is able to infect mice, while *T. c. marinkellei* is not. Tcbat has also been detected in a Colombian child as well as in pre-Columbian mummies in Chile.

Members of several bat families have been found to harbor Tcbat: Phyllostomidae, Vespertilionidae, and Noctilionidae. TcbI inhabits Thyropteridae, Noctilionidae, Phyllostomidae, Emballonuridae, and Molossidae, while TcbII inhabits Phyllostomidae, Vespertilionidae, and Mormoopidae. The bat hosts include insectivorous, frugivorous, nectivorous, and carnivorous species (Lima *et al*. 2015a). TcbII appears to be the most basal lineage. Tcbat and TcbI are sister groups and TcbII–TcbVI form a distinct clade (Pinto *et al*. 2012; Lima *et al*. 2015a).

12.2.4.2 T. c. marinkellei *T. c. marinkellei* has a greater degree of nucleotide diversity than any of the *T. cruzi* genotypes associated with human disease. Such a high degree of diversity of *T. c. marinkellei* suggests that *T. cruzi* and bats have a long evolutionary history and that bats may be the original hosts of this parasite (Pinto *et al*. 2015).

T. erneyi from African bats is closely related to *T. c. marinkellei* from the Americas (Lima *et al*. 2012). A new species of trypanosome, *Trypanosoma livingstonei*, was isolated by hemoculture from *Rhinolophus landeri* and *Hipposideros caffer* from Mozambique in southeast Africa. This new species has unique morphological and ultrastructural features as well as growth behavior (the trypomastigote form does not infect HeLa cells). Phylogenetic inferences suggest that *T. livingstonei* is at the edge of the *T. cruzi* clade (Lima *et al*. 2013).

12.2.4.3 T. cruzi vectors In Ecuador, some of the triatomine vectors of *T. cruzi, Cavernicola pilosa* and *Triatoma dispar*, share shelters with bats. By contrast, *T. c. marinkellei* uses members of the *Cavernicola* genus exclusively as its vector. Members of this insect genus are typically associated with bat colonies. Triatomes dwelling in tree

holes and caves, palms, and house roofs transmit *T. cruzi* among bats. The vectors for Tcbat are not currently known, but this parasite does not develop in *Triatoma infestans* or *Rhodnius prolixus*, two of the major vectors for Chagas disease in humans. Cimicidae serve as the vectors of *T. dionisii* in Europe, while bat bugs of the closely related Polyctenidae are commonly associated with African bats and may act as the vectors of *T. erneyi. R. prolixus* readily feed upon some neotropical bat species, two of which can be infected after being bitten by *R. prolixus* infected with *T. rangeli. Carollia, Glossophaga*, and *Molossus* bat species are also infected by ingesting *T. cruzi*- or *T. rangeli*-infected triatomines or by subcutaneous or intragastic inoculation with infected fecal suspensions. Other potential vectors include dipterans, ticks, mites, and fleas that parasitize bats (Lima *et al*. 2012).

12.3 *LEISHMANIA*

The life cycle of *Leishmania* parasites in humans begins with the bite of infected female phlebotomine sandflies during a blood meal. The infective promastigote stage enters people via the wound site before being phagocytized by dermal macrophages. Within these cells of the innate immune system, promastigotes transform into the nonflagellated, amastigote stage and divide. The resulting progeny undergo multiple rounds of infecting new phagocytic cells until an infected cell is ingested by another female sandfly. The amastigotes then transform into the flagellated promastigotes, which develop and divide in the insect's gut before travelling to the proboscis for transmission to the next mammalian host (CDC 2016c).

12.3.1 *Leishmania* and disease

As of 2015, leishmaniasis was endemic in 98 countries and territories and is absent only in Australia and Antarctica. More than 1 million new cases are reported each year, with the number of cases increasing in many regions. *Leishmania* species cause several human diseases, including cutaneous, mucocutaneous, and visceral leishmaniasis, the most severe form that, with its mortality rate of 10%, is the second leading cause of death from tropical parasitic infections (reviewed by Handler *et al*. 2015).

Cutaneous leishmaniasis is evenly distributed throughout Western Asia, the Mediterranean region, and Latin America and is now endemic in parts of Texas and, perhaps, Oklahoma. It is the mildest, self-resolving form of the disease, but the resulting ulcers on the face and extremities may be disfiguring. Mucocutaneous leishmaniasis is found in Latin America and may occur years after recovering from the cutaneous form of the disease. It is characterized by mucosal erosions or inflammation that may lead to perforation of the nasal septum and severe damage to the mouth, nose, and pharynx. The visceral form of the disease is found primarily in India, Bangladesh, South Sudan, Sudan, Brazil, and Ethiopia. It is associated with fever, weight loss, hepatosplenomegaly, and destruction of internal organs, particularly the spleen, liver, and bone marrow (reviewed by Handler *et al*. 2015). Development of visceral disease may result from viral infection of the *Leishmania* protozoan: *Leishmania* RNA virus-1 in *L. brasiliensis* and *L. guyanensis* in the Americas and, elsewhere, *Leishmania* RNA virus-2 in *L. major*. Recognition of the virus by the host's Toll-like receptor 3 may lead to the destruction of the *Leishmania*

parasite, dispersing the virus and triggering a hyperinflammatory reaction involving pro-inflammatory cytokines and chemokines (reviewed by Handler *et al*. 2015).

Visceral leishmaniasis is associated with infection by any of several *Leishmania* species: *Leishmania donovani* (India, Bangladesh, Nepal, and Pakistan); *Leishmania infantum chagasi* (Europe and Africa), and *Leishmania chagasi* in the Americas. South American *Leishmania* species have been placed into the subgenera *Leishmania* or *Viannia*. The former cause visceral disease, and the latter, cutaneous and mucocutaneous diseases. Vertebrate hosts include humans, domestic dogs, and wild mammals. While the primary reservoir hosts are dogs and wild canids, forest rodents, sloths, opossums, cats, and bats also serve as reservoirs (reviewed in Acosta *et al*. 2014). The vectors for this parasite are female phlebotomine sandflies of the *Lutzomyia* genus in the New World or *Phlebotomus* in the Old World. The sandflies bite many animal species, including humans and bats (Handler *et al*. 2015).

12.3.2 *Leishmania* and bats

12.3.2.1* Leishmania *and bats in the Americas A study of over 650 bats of 28 species in southwestern Brazil found antibodies against *Leishmania* in 0.9% of the bats in non-urban areas. Additionally, *Leishmania (Leishmania) amazonensis* DNA was detected in 18 bats and *L. (Leishmania) i. chagasi* DNA in 3 bats (Savani *et al*. 2010). A recent survey of 25 species of wild and 2 species of domestic animals in Brazil did not find evidence of infection with *L. i. chagasi* in tested domestic animals (dogs and horses) (Acosta *et al*. 2014). Only bats were found to be infected by this parasite (Acosta *et al*. 2014). The *Leishmania*-infected bat species in this and other studies include *M. molossus, A. lituratus, P. lineatus*, and *Glossophaga soricina*. A separate study in southwestern Brazil found *Leishmania (Viannia) braziliensis* in two bats (Shapiro *et al*. 2013). Kinetoplast DNA (kDNA) from *Leishmania* was present in 23.9% of urban bats in Brazil and trypanosome DNA in 3.9%. Among the Leishmania species, *L. amazonensis* comprised 78.3% of the samples, *L. infantum* was 17.4%, and 4.3% were dual-infected with *L. infantum* and *L. amazonensis*. DNA of these two *Leishmania* species was also present in *D. rotundus* (de Olivera *et al*. 2015).

Berzunza-Cruz *et al*. (2015) studied over 400 bats from southeastern Mexico, a site of endemic zoonosis for cutaneous leishmaniosis caused by *Leishmania mexicana*. Some of the foci of infection are in shade-grown cocoa and coffee plantations or near forests that serve as breeding grounds for sandflies. A variety of bat species also inhabit these areas, living off of the abundant fruits. Both sandflies and bats inhabit the caves and crevices in the study region. Thirteen species of bats (9.8% of the tested animals) were PCR-positive for *L. mexicana* DNA. Infected tissues included skin, heart, and liver. All infected bats were netted in the Gulf Coastal Plain, a "hotspot" for leishmaniosis. Additionally, almost all infected animals belong to the Phyllostomidae family: *A. jamaicensis* (6% prevalence, $n=86$), *A. lituratus* (7%, $n=41$), *Dermanura phaeotis* (8%, $n=37$), *Carollia sowelli* (4%, $n=45$), *Choeroniscus godmani* (23%, $n=13$), *D. rotundus* (7%, $n=14$), *Glossophaga commissarisi* (75%, $n=8$), *G. soricina* (27%, $n=26$), *Leptonycteris curasoae* (70%, $n=2$), *P. discolor* (100%, $n=1$), *Sturnira lilium* (11%, $n=63$), and *Sturnira ludovici* (4%, $n=25$). Additionally, 25% of *Pteronotus personatus* ($n=4$) of the Mormoopidae family was infected. There was no evidence of macroscopic lesions in the infected bat tissues. Parasites isolated from two experimentally infected bats were able to productively

infect mice, demonstrating that *L. mexicana* is able to survive in an infectious form after exposure to the bat immune system (Berzunza-Cruz *et al*. 2015).

In Venezuela, *L. i. chagasi* was isolated from Seba's short-tailed bats (*C. perspicillata*) in an area without human visceral *Leishmania* cases and few cases of the cutaneous form of the disease. These parasites were able to infect the footpad of inoculated mice and cause the typical nodular lesion (De Lima *et al*. 2008). No infected bat tissue (skin, liver, wing membrane, nasal mucosa) was detected by PCR from 216 bats representing 29 species, in regions of French Guiana reporting high incidences of human cutaneous cases. In the latter study area, several *Leishmania* species have been shown to be present: *Leishmania guyanensis, L. amazonensis, L. braziliensis, Leishmania naiffi,* and *Leishmania lainsoni* (Rotureau *et al*. 2006).

12.3.2.2 **Leishmania *and bats in Europe*** Leishmaniasis is hyperendemic in northeast Spain and dogs are known to be an important reservoir host. Information concerning the role of wildlife in the area as disease reservoirs is sparse. When blood from 35 Schreibers' bats (*Miniopterus schreibersii*) was tested for *L. donovani* group kDNA, however, all samples were PCR-negative (Millán *et al*. 2014). This might be a result of limited sample size or from an absence of the parasite in this species of bat in this area of Spain.

12.3.2.3 **Leishmania *and bats in Africa*** Leishmaniasis is also endemic in Africa. Wild and domestic mammals, such as dogs, rodents, and rock hyraxes in Ethiopia are known to host *Leishmania* species. In order to determine whether bats also are infected by members of this group of parasites, DNA from the spleens of 163 bats of 23 species and 18 genera was analyzed by PCR for kDNA. Eight of the bats were positive: four were from endemic areas and the remaining four were from regions that are non-endemic for leishmaniasis in humans. *Leishmania tropica* was isolated from *Cardioderma cor* and *Leishmania major* was isolated from *Nycteris hispida* (Kassahun *et al*. 2015). Even though these leishmanial species cause human cutaneous disease, no dermal lesions were seen on the infected bats.

12.4 CONCLUSIONS

Members of *Trypanosoma* and *Leishmania* genera are blood-borne protozoan parasites that infect a very wide variety of hosts, including vertebrates and invertebrates. Some species from each genus cause mild to life-threating diseases in humans. The latter, severe diseases include Chagas disease, African sleeping sickness, and visceral leishmaniasis (caused by *T. c. cruzi, T. b. gambiense* and *T. b. rhodesiense*, and several *Leishmania* species, respectively). As is the case for many parasitic protozoans, their life cycles include several life stages in several hosts, including humans, dogs, opossums, and chickens, in addition to bats. They are typically transmitted to the vertebrate host by the saliva or feces of hematophagous arthropods.

Approximately 100 bat species are infected by trypanosomes throughout the world. Many of these parasites are either bat-specific or are not pathogenic to humans. Most bats infected by trypanosomes are insectivorous and are exposed to infected insects during feeding. Most trypanosomes present in bats in the Americas are of the *Schizotrypanum* subgenus and

reside in skeletal, cardiac, or smooth muscles for years. This subgenus species typically restricts their mammalian hosts to bats, except for the generalist, *T. c. cruzi*. A variety of bat species throughout Latin America, in addition to other vertebrates, are infected by *T. c. cruzi*, which is highly pathogenic for humans. In the southern US, domestic and wild canids, wild felines, skunks, raccoons, and rodents are infected by *T. c. cruzi*. Of note, the prevalence of infection in bats is very low in comparison with that found in other animals, suggesting that bats may not be a significant *T. cruzi* reservoir in this area. Additionally, several trypanosome species in bats are members of the *Herpetosoma* subgenus, including *T. rangeli*, which has only infected a small number of humans. At least one species of trypanosome, *Trypanosoma teixeirae* from Australia, is pathogenic to bats. Several life stages of this parasite were found in the flying fox *P. scapulatus* in a moribund bat.

Leishmania species also have a wide distribution and are the causative agents for several human diseases, including the fairly mild cutaneous to severe or life-threatening mucocutaneous and visceral leishmaniasis. Visceral leishmaniasis results from infection by *L. donovani, L. infantum chagasi*, and *L. chagasi*. The primary reservoir hosts are dogs and wild canids; however, other animals, may also act as reservoir hosts. Sandflies act as *Leishmania* vectors.

L. i. chagasi has been found in bats in South America and a 2014 report from Brazil (Acosta *et al*. 2014) detected this agent of human visceral leishmaniasis only in bats and not in 25 species of wild animals, dogs, or horses. In an area of Spain in which visceral leishmaniasis is hyperendemic, dogs are an important reservoir host of *L. donovani*. *M. schreibersii* bats from the region ($n = 35$) were PCR-negative for *Leishmania*. Given the small sample size and the testing of only one bat species, this does not, nevertheless, necessarily mean that Spanish bat populations may not also be infected with and serve as an additional reservoir for *Leishmania* species.

Thirteen species of Mexican bats, almost all belonging to the Phyllostomidae family, harbor agents of cutaneous leishmaniasis. In Ethiopia, dogs, rodents, and rock hyraxes are infected by *Leishmania* species responsible for cutaneous disease in humans. Eight of 23 tested bat species were PCR-positive for kinetoplast DNA, however, four of these came from regions which are not endemic for human disease.

In conclusion, many species of bats harbor *Trypanosoma* or *Leishmania* species, some of which are specific for bats. Some bats also harbor kinetoplastid species which infect humans and cause mild, transient disease, while other parasite species present in bats cause severe visceral disease in humans. While dogs and wild canids are major reservoirs for human disease, bats may also act as reservoir hosts, especially for *L. i. chagasi* in some areas of Brazil. More work needs to be done in order to determine which sandfly species act as vectors for the bats in those areas in order to determine whether these species of insects also feed upon humans.

REFERENCES

Acosta IdCL, da Costa AP, Gennari SM, Marcili A. 2014. Survey of *Trypanosoma* and *Leishmania* in wild and domestic animals in an Atlantic rainforest fragment and surroundings in the state of Espírito Santo, Brazil. *Journal of Medical Entomology.* 51(3):686–693.

Austen JM, O'Dea M, Jackson B, Ryan U. 2015. High prevalence of *Trypanosoma vegrandis* in bats from Western Australia. *Veterinary Parasitology.* 214(3–4):342–347.

Baker JR, Miles MA, Godfrey DG, and Barrett TV. 1978. Biochemical characterization of some species of *Trypanosoma* (*Schizotrypanum*) from bats (Microchiroptera). *American Journal of Tropical Medicine and Hygiene.* 27(3):483–491.

Barbosa AD, Mackie JT, Stenner R, Gillett A, Irwin P, Ryan U. 2016. *Trypanosoma teixeirae*: A new species belonging to the *T. cruzi* clade causing trypanosomosis in an Australian little red flying fox (*Pteropus scapulatus*). *Veterinary Parasitology*. 223:214–221.

Barnabe C, Brisse S, Tibayrenc M. 2003. Phylogenetic diversity of bat trypanosomes of subgenus *Schizotrypanum* based on multilocus enzyme electrophoresis, random amplified polymorphic DNA, and cytochrome *b* nucleotide sequence analyses. *Infection, Genetics and Evolution.* 2:201–208.

Berzunza-Cruz M, Rodríguez-Moreno Á, Gutiérrez-Granados G, González-Salazar C, Stephens CR, Hidalgo-Mihart M, Marina CF, Rebollar-Téllez EA, Bailón-Martínez D, Balcells CD, Ibarra-Cerdeña CN, Sánchez-Cordero V, Becker I. 2015. *Leishmania (L.) mexicana* infected bats in Mexico: novel potential reservoirs. *PLoS Neglected Tropical Diseases*. 9(1):e0003438.

Cavazzana M Jr, Marcili A, Lima L, da Silva FM, Junqueira ACV, Veludo HH, Viola LB, Campaner M, Nunes VLB, Paiva F, Coura JR, Camargo EP, Teixeira MMG. 2010. Phylogeographical, ecological and biological patterns shown by nuclear (ssRNA and gGAPDH) and mitochondrial (Cyt b) genes of trypanosomes of the subgenus *Schizotrypanum* parasitic in Brazilian bats. *International Journal for Parasitology.* 40:345–355.

CDC. 2016a. Parasites – American Trypanosomiasis (also known as Chagas Disease). http://www.cdc.gov/parasites/chagas/index.html. Accessed November 7, 2016.

CDC. 2016b. Parasites – African Trypanosomiasis (also known as Sleeping Sickness). http://www.cdc.gov/parasites/sleepingsickness/index.html. Accessed November 8, 2016.

CDC. 2016c. Parasites – Leishmaniasis. https://www.cdc.gov/parasites/leishmaniasis/index.html. Accessed November 8, 2016.

Cottontail VM, Kalko EKV, Cottontail I, Wellinghausen N, Tschapka M, Perkins SL, Pinto CM. 2014. High local diversity of *Trypanosoma* in a common bat species, and implications for the biogeography and taxonomy of the *T. cruzi* clade. *PLoS ONE*. 9(9):e108603.

Cottontail VM, Wellinghausen N, Kalko EKV. 2009. Habitat fragmentation and haemoparasites in the common fruit bat, *Artibeus jamaicensis* (Phyllostomidae) in a tropical lowland forest in Panamá. *Parasitology.* 136:1133–1145.

Curtis-Robles R, Wozniak EJ, Auckland LD, Hamer GL, Hamer SA. 2015. Combining public health education and disease ecology research: using citizen science to assess Chagas disease entomological risk in Texas. *PLoS Neglected Tropical Diseases.* 9:e0004235.

da Costa AP, Nunes PH, Leite BHS, da S. Ferreira JIG, Tonhosolo R, da Rosa AR, da Rocha PA, Aires CC, Gennari SM, Marcili A. 2016. Diversity of bats trypanosomes in hydroeletric (sic) area of Belo Monte in Brazilian Amazonia. *Acta Tropica.* 164:185–193.

da Silva FM, Marcili A, Lima L, Cavazzana M Jr, Ortiz PA, Campaner M, Takeda GF, Paiva F, Nunes VLB, Camargo EP, Teixeira MMG. 2009. *Trypanosoma rangeli* isolates of bats from Central Brazil: Genotyping and phylogenetic analysis enable description of a new lineage using spliced-leader gene sequences. *Acta Tropica.* 109:199–207.

De Lima H, Rodríguez N, Barrios MA, Ávila Á, Cañizales I, Gutiérrez S. 2008. Isolation and molecular identification of *Leishmania chagasi* from a bat (*Carollia perspicillata*) in northeastern Venezuela. *Mem Inst Oswaldo Cruz, Rio de Janeiro.*103(4):412–414.

de Oliveira FM, Costa LHC, de Barros TL, Ito PKRK, Colombo FA, de Carvalho C, Pedro WA, Queiroz LH, Nunes CM. 2015. First detection of *Leishmania* spp. DNA in Brazilian bats captured strictly in urban areas. *Acta Tropica*. 150:176–181.

García L, Ortiz S, Osorio G, Torrico MC, Torrico F, Solari A. 2012. Phylogenetic analysis of Bolivian bat trypanosomes of the subgenus *Schizotrypanum* based on cytochrome b sequence and minicircle analyses. *PLoS ONE.* 7(5):e36578.

Gardner RA, Molyneux DH. 1988. Schizotrypanum in British bats. *Parasitology.* 97(Pt 1):43–50.

Gorchakov R, Trosclair LP, Wozniak EJ, Feria-Arroyo PT, Garcia MN, Gunter SM, Murray KO. 2016. *Trypanosoma cruzi* infection prevalence and bloodmeal analysis in triatomine vectors of Chagas disease from rural peridomestic locations in Texas, 2013-2014. *Journal of Medical Entomology*. DOI: http://dx.doi.org/10.1093/jme/tjw040.

Grisard EC, Sturm NR, Campbell DA. 2003. A new species of trypanosome, *Trypanosoma desterrensis* sp. n., isolated from South American bats. *Parasitology*. 127(Pt 3):265–271.

Hamilton PB, Teixeira MMG, Steven JR. 2012. The evolution of *Trypanosoma cruzi*: the 'bat seeding' hypothesis. *Trends in Parasitology.* 28(4):136–141.

Handler MZ, Patel PA, Kapila R, Al-Qubati Y, Schwartz RA. 2015. Cutaneous and mucocutaneous leishmaniasis. Clinical perspectives. *Journal of the American Academy of Dermatology.* 73(6):897–908.

Hoare CA. 1972. *The Trypanosomes of Mammals*. Blackwell Scientific Publications: Oxford.

Hodo CL, Goodwin CC, Mayes BC, Mariscal JA, Waldrup KA, Hamer SA. 2016. Trypanosome species, including *Trypanosoma cruzi*, in sylvatic and peridomestic bats of Texas, USA. *Acta Tropica*. 164:259–266.

Kassahun A, Sadlova J, Benda P, Kostalova T, Warburg A, Hailu A, Baneth G, Volf P, Votypka J. 2015. Natural infection of bats with *Leishmania* in Ethiopia. *Acta Tropica.* 150:166–170.

Kjos SA, Snowden KF, Olson JK. 2009. Biogeography and *Trypanosoma cruzi* infection: prevalence of Chagas disease vectors in Texas, USA. *Vector Borne Zoonotic Diseases*. 9:41–50.

Lima L, da Silva FM, Neves L, Attias M, Takata CSA, Campaner M, de Souza W, Hamilton PB, Teixeira MMG. 2012. Evolutionary insights from bat trypanosomes: morphological, developmental and phylogenetic evidence of a new species, *Trypanosoma* (*Schizotrypanum*) *erneyi* sp. nov., in African bats closely related to *Trypanosoma (Schizotrypanum*) *cruzi* and allied species. *Protist.* 163:856–872.

Lima L, Espinosa-Álvarez O, Hamilton PB, Neves N, Marta Campaner CSA, Attias M, de Souza W, Camargo EP, Teixeira MMG. 2013. *Trypanosoma livingstonei*: a new species from African bats supports the bat seeding hypothesis for the *Trypanosoma cruzi* clade. *Parasites & Vectors.* 6:221.

Lima L, Espinosa-Álvarez O, Ortiz PA, Trejo-Varón JA, Carranza JC, Pinto CM, Serrano MG, Buck GA, Camargo EP, Teixeira MMG. 2015a. Genetic diversity of *Trypanosoma cruzi* in bats, and multilocus phylogenetic and phylogeographical analyses supporting Tcbat as an independent DTU (discrete typing unit). *Acta Tropica.* 151:166–177.

Lima L, Espinosa-Álvarez O, Pinto CM, Cavazzana M Jr, Pavan AC, Carranza JC, Lim BK, Campaner M, Takata CSA, Camargo EP, Hamilton PB, Teixeira MMG. 2015b. New insights into the evolution of the *Trypanosoma cruzi* clade provided by a new trypanosome species tightly linked to Neotropical *Pteronotus* bats and related to an Australian lineage of trypanosomes. *Parasites & Vectors.* 8:657.

Lisboa CV, Pinho AP, Herrera HM, Gerhardt M, Cupolillo E, Jansen AM. 2008. *Trypanosoma cruzi* (kinetoplastida, Trypanosomatidae) genotypes in neotropical bats in Brazil. *Veterinary Parasitology.* 156:314–318.

Marcili A, Lima L, Cavazzana M Jr, Junqueira ACV, Veludo HH, Da Silva FM, Campaner M, Paiva F, Nunes VLB, Teixeira MMG. 2009. A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and histone H2B genes and genotyping based on ITS1 rDNA. *Parasitology.* 136:641–655.

Marinkelle CJ. 1977. *Trypanosoma (Herpetosoma) longiflagellum* sp. n. from the tomb bat, *Taphozous nudiventris*, from Iraq. *Journal of Wildlife Diseases*. 13(3):262–264.

Millán J, López-Roig M, Cabezón O. 2014. Absence of *Leishmania infantum* in cave bats in an endemic area in Spain. *Parasitology Research.* 13:1993–1995.

Pinto ADS, Bento DNDC. 1986. *Trypanosoma cruzi*-like bloodstream trypomastigotes in bats from the state of Piauí, Northeastern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical.* 19(1):31–34.

Pinto C, Kalko EKV, Cottontail I, Wellinghausen N. Cottontail VM. 2012. TcBat: a bat-exclusive lineage of *Trypanosoma cruzi* in the Panama Canal Zone, with comments on its classification and the use of the 18S rRNA gene for lineage identification. *Infection, Genetics and Evolution.* 12:1328–1332.

Pinto CM, Ocaña-Mayorga S, Tapia EE, Lobos SE, Zurita AP, Aguirre-Villacís F, MacDonald A, Villacís AG, Lima L, Teixeira MMG, Grijalva MJ, Perkins SL. 2015. Bats, trypanosomes, and triatomines in Ecuador: new insights into the diversity, transmission, and origins of *Trypanosoma cruzi* and Chagas disease. *PLoS ONE.* 10(10):e0139999.

Ramírez JD, Tapia-Calle G, Muñoz-Cruz G, Poveda C, Rendón LM, Hincapié E, Guhl F. 2014. Trypanosome species in neo-tropical bats: Biological, evolutionary and epidemiological implications. *Infection, Genetics and Evolution.* 22:250–256.

Rotureau B, Catzeflis F, Carme B. 2006. Absence of *Leishmania* in Guianan bats. *American Journal of Tropical Medicine and Hygiene.* 74(2):318–321.

Savani ESMM, de Almeida MF, de Oliveira Camargo MCG, D'Auria SRN, Silva MMS, de Oliveira ML, Sacramento D. 2010. Detection of *Leishmania (Leishmania) amazonensis* and *Leishmania (Leishmania) infantum chagasi* in Brazilian bats. *Veterinary Parasitology.* 168:5–10.

Shapiro JT, da Costa Lima Junior MS, Dorval MEC, de Oliveira Franc A, Cepa Matos MDF, Bordignon MO. 2013. First record of *Leishmania braziliensis* presence detected in bats, Mato Grosso do Sul, southwest Brazil. *Acta Tropica* 128:171–174.

Steindel M, Grisard EC, de Carvalho, Pinto CJ, Cordeiro FD, Ribeiro-Rodrigues R, Romanha AJ. 1998. Characterization of trypanosomes from the subgenus *Schizotrypanum* isolated from bats, *Eptesicus* sp. (Chiroptera: Vespertilionidae), captured in Florianópolis, Santa Catarina State, Brazil. *Journal of Parasitology*. 84(3):601–607.

Thomas ME, Rasweiler IV JJ, D'Alessandro A. 2007. Experimental transmission of the parasitic flagellates *Trypanosoma cruzi* and *Trypanosoma rangeli* between triatomine bugs or mice and captive neotropical bats. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro.* 102(5):559–565.

Thompson CK, Godfrey SS, Thompson RCA. 2014. Trypanosomes of Australian mammals: a review. *International Journal of Parasitology: Parasites and Wildlife.* 3:57–66.

Tulane University. 2016. Kinetoplastids. http://www.tulane.edu/~wiser/protozoology/notes/kinet.html#tr_vs_tg. Accessed November 7, 2016.

V

FUNGAL INFECTIONS OF BATS

13

WHITE-NOSE SYNDROME AND BATS

13.1 INTRODUCTION TO *PSEUDOGYMNOASCUS DESTRUCTANS*

Pseudogymnoascus destructans belongs to the fungal phylum Ascomycota, class Leotiomycetes, that includes several important plant pathogens, but no other major animal pathogens. *P. destructans* reproduces asexually, via conidia at the end of long, branched conidiophores bearing unique, asymmetrically curve-shaped conidia that may reinfect the same individual, be transmitted to a different host, or be released into the environment (Gargas *et al.* 2009). The fungus is adapted to the cold, humid conditions present in hibernacula, only replicating at temperatures below 20 °C, with an optimal range of 5–10 °C (Blehert *et al.* 2009; Chaturvedi *et al.* 2010). The critical upper temperatures for *in vitro* growth are 19.0–19.8 °C. Morphological changes are seen at temperatures above 12 °C and, above 15 °C, hyphae are deformed, thicker, and the tips have a branched, antler-like morphology. Conidia are pyriform to globoid in shape at these higher temperatures and colony morphology changes from a white, smooth appearance to tan to dark brown and the colonies are heavily creased (Verant *et al.* 2012). Eleven *Geomyces* isolates were obtained from the wings of hibernating bats. All of these strains were psychrotolerant, except for *Geomyces destructans* (now *P. destructans*), which is psychrophilic (Johnson *et al.* 2013).

Low, stable low temperatures are the sole factor typically beneficial for development of psychrophilic microorganisms, such as *P. destructans*. Accordingly, fungi commonly grow on organic matter in underground environments. Their spores are carried by water, air currents, and animals (bats, arthropods) and humans. A study of a large, man-made underground bat reserve in Poland found that the external environment and air currents

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are believed to be the major determining factors of numbers and species composition of underground airborne fungi, most of which are located in the twilight zone or near entrances or ventilation shafts (reviewed by Kokurewicz *et al.* 2016).

In a study of sediment samples from hibernacula in North America, viable *P. destructans* were recovered from half of the sites sampled during late summer ($n = 14$), despite the absence of hibernating bats for many months. See Table 13.1 for a list of bats currently known to be infected by *P. destructans.* One of the positive sites had not been occupied by bats for 2 years (Lorch *et al.* 2013b). The year-long cool temperatures of caves and underground mines provide an ideal environment for the persistence of the fungus long-term, even in the absence of its host. It would be useful to conduct further studies on sediments in the late autumn, just prior to usage by hibernating bats. Such work might also find characteristics of the portions of hibernacula that are most likely to support *P. destructans*, including the ceiling areas where the roosting bats have a higher chance of encountering the fungus (Lorch *et al.* 2013b).

Although bat-to-bat transmission appears to be the major route by which bats become infected by *P. destructans*, arthropods may also be indirectly involved in transmission between bats. Vanderwolf *et al.* (2016) isolated 87 fungal taxa from 64 arthropod species from entrances from *P. destructans*-positive underground mines or caves in Canada. Viable *P. destructans* was cultured from 15.3% of the arthropods, most commonly from harvestmen (*Nelima elegans*), which form large aggregates. Other fungi were detected deeper in the caves. Arthropods also, however, play a role in controlling cave fungi by producing antifungal compounds and by consuming fungi. Further work is needed to determine the numbers of *P. destructans*-harboring arthropods found deeper in the caves, even though some bats do reside near cave entrances during autumn swarming. A separate study detected *P. destructans* on the surface of all tested hematophagous wing mites (*Spinturnix myoti*) ($n = 33$) taken from the *P. destructans*-positive *M. myotis* at the end of the hibernation period in a European site (Lučan *et al.* 2016). Fungal load on the mites correlated with that found on the bats. These mites switch hosts horizontally by crawling between bats and may thus mechanically transmit *P. destructans* within a site.

13.2 WHITE-NOSE SYNDROME

White-nose syndrome (WNS) is a widespread, epizootic disease that causes a fatal illness in 30–99% of hibernating bats infected by *P. destructans* in North America. This fungus causes lesions containing fungal hyphae densely packed in cupping erosions in the epidermis and may invade the dermis as well (Meteyer *et al.* 2009). Hyphae penetrate the connective tissue of skin devoid of hair, including cutaneous tissues of the ears and wings and in hair follicles and sebaceous and apocrine glands of the muzzle in the absence of inflammation in hibernating bats. Severe wing damage with a robust suppurative inflammatory response has been seen, however, in bats that have recently emerged from hibernation (Meteyer *et al.* 2009; Cryan *et al.* 2010).

Infection is seasonal, with bats becoming transiently infected in the autumn. Transmission among bats rapidly increases as hibernation begins in early winter, infection intensity peaks by late winter with nearly all of the individuals infected, before clearing by the summer (Langwig *et al.* 2015a). Multiple factors are involved in the

TABLE 13.1 Bat species reported to be infected by *Pseudogymnoascus destructans*

Bat family	Bat common name	Bat species	Microbe
Vespertilionidae	Western barbastelle	*Barastella barastellus*	*Pseudogymnoascus destructans*
Vespertilionidae	Rafinesque's big-eared bat	*Corynorhinus rafinesquii*	*Pseudogymnoascus destructans*
Vespertilionidae	Virginia big-eared bat	*Corynorhinus townsendii virginianus*	*Pseudogymnoascus destructans*
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Pseudogymnoascus destructans*
Vespertilionidae	Northern bat	*Eptesicus nilssonii*	*Pseudogymnoascus destructans*
Vespertilionidae	Silver-haired bat	*Lasionycteris noctivagans*	*Pseudogymnoascus destructans*
Vespertilionidae	Eastern red bat	*Lasiurus borealis*	*Pseudogymnoascus destructans*
Vespertilionidae	Greater tube-nosed bat	*Murina leucogaster*	*Pseudogymnoascus destructans*
Vespertilionidae	Ussuri tube-nosed	*Murina ussuriensis*	*Pseudogymnoascus destructans*
Vespertilionidae	Alcathoe myotis	*Myotis alcathoe*	*Pseudogymnoascus destructans*
Vespertilionidae	Southeastern bat	*Myotis austroriparius*	*Pseudogymnoascus destructans*
Vespertilionidae	Bechstein's bat	*Myotis bechsteinii*	*Pseudogymnoascus destructans*
Vespertilionidae	Lesser mouse-eared bat	*Myotis blythii*	*Pseudogymnoascus destructans*
Vespertilionidae	Brandt's bat	*Myotis brandtii*	*Pseudogymnoascus destructans*
Vespertilionidae	Large myotis	*Myotis chinensis*	*Pseudogymnoascus destructans*
Vespertilionidae	Pond bat	*Myotis dasycneme*	*Pseudogymnoascus destructans*
Vespertilionidae	Daubenton's myotis	*Myotis daubentonii*	*Pseudogymnoascus destructans*
Vespertilionidae	Geoffroy's bat	*Myotis emarginatus*	*Pseudogymnoascus destructans*
Vespertilionidae	Gray bat	*Myotis grisescens*	*Pseudogymnoascus destructans*
Vespertilionidae	Eastern small-footed myotis	*Myotis leibii*	*Pseudogymnoascus destructans*
Vespertilionidae	Little brown bat	*Myotis lucifugus*	*Pseudogymnoascus destructans*
Vespertilionidae	Big-footed myotis	*Myotis macrodactylus*	*Pseudogymnoascus destructans*
Vespertilionidae	Large mouse-eared bat	*Myotis myotis*	*Pseudogymnoascus destructans*
Vespertilionidae	Whiskered bat	*Myotis mystacinus*	*Pseudogymnoascus destructans*
Vespertilionidae	Natterer's bat	*Myotis nattereri*	*Pseudogymnoascus destructans*
Vespertilionidae	Eastern water bat	*Myotis petax*	*Pseudogymnoascus destructans*
Vespertilionidae	Northern long-eared myotis	*Myotis septentrionalis*	*Pseudogymnoascus destructans*
Vespertilionidae	Indiana bat	*Myotis sodalis*	*Pseudogymnoascus destructans*
Vespertilionidae	Cave myotis	*Myotis velifer*	*Pseudogymnoascus destructans*
Vespertilionidae	Eastern pipestrelle	*Perimyotis subflavus*	*Pseudogymnoascus destructans*
Vespertilionidae	Brown long-eared bat	*Plecotus auritus*	*Pseudogymnoascus destructans*
Rhinolophidae	Greater horseshoe bat	*Rhinolophus ferrumequinum*	*Pseudogymnoascus destructans*
Rhinolophidae	Lesser horseshoe bat	*Rhinolophus hipposideros*	*Pseudogymnoascus destructans*

epidemiology of WNS, including the length of hibernation season, microclimatic conditions (better survival in coldest and driest roosts), type of the hibernaculum, and solitary versus gregarious hibernation behavior (Langwig *et al.* 2012). Of note, declining numbers of individuals in the gregarious species lead to decreases in social group size, thus reducing the likelihood of extinction. Since *P. destructans* is transmitted between bats via direct contact rather than inhalation, densely packed populations in large hibernaculum may increase contact and thus bat-to-bat transmission. In a study of naturally infected, captive *M. lucifugus*, sex and temperature affected survival, with increased survival in males and in bats housed at a lower temperature (4 versus 7 or 10 °C) (Grieneisen *et al.* 2015), perhaps since the optimal temperature range for the fungus is 5–10 °C (Blehert *et al.* 2009), with slower growth occurring at the lower temperatures (Verant *et al.* 2012). This is in agreement with the finding that WNS-affected hibernacula in warmer regions have greater population decline than those found in colder regions (Langwig *et al.* 2012). Using experimentally infected *M. lucifugus*, Johnson *et al*. (2015) also found that lower temperatures increase the chance of bat survival, but reported a higher rate of death in females. The differences in these studies may be due to naturally versus experimentally infected animals or relate to the number of fungi infecting the different groups of bats.

13.2.1 Arousal, loss of fat reserves, and dehydration

In order to survive the lack of available food in the winter, bats increase their body fat reserves in late summer. In the case of *M. lucifugus*, the fat stores increase from approximately 7 to 27% of total body mass (Reeder *et al.* 2012). *P. destructans* infection leads to repeated arousals during the winter hibernation, increasing bats' metabolic activity, and depleting their white and brown fat reserves. White fat serves as an energy storing tissue, while brown fat tissue is involved in heat generation. Each arousal depletes about 5% of the winter energy budget and shortens the time a bat may hibernate by approximately 9 days (Thomas *et al.* 1990).

Infected bats often leave their hibernation sites during late winter emaciated and dehydrated in order to search for food, usually leading to their deaths (Bat Conservation International 2017). Mass-specific normothermic evaporative water loss is greater in uninfected individuals of the highly *P. destructans*-susceptible *M. lucifugus* species in comparison with that occurring in *M. nattereri*, a species that has not demonstrated significant mortality from WNS. Dataloggers attached to the backs of 83 free-ranging little brown bats (*M. lucifugus*) in hibernacula in the northeastern US demonstrated that bats with WNS underwent arousal to euthermic body temperature more often than uninfected bats. Increased arousal appears to be responsible for the high fatality rate due to increased energy expansion necessary for the bats to survive over the winter and the number of arousals predicted date of death and correlated with severity of *P. destructans* infection (Blehert 2012; Reeder *et al.* 2012).

Dehydration in *P. destructans*-infected bats may cause them to arouse from torpor in order to drink (Willis *et al.* 2011). Dehydration has also been implicated as the best predictor of bat arousal frequency (Thomas & Cloutier 1992), thus dehydration may play an important role in *P. destructans*-induced arousal pathology (Willis *et al.* 2011). Due to the scarcity of data concerning evaporative water loss during normothermic and torpor states, more research is needed to confirm these findings. Damage to the wing surface,

however, leads to fluid loss, similar to that seen in burn victims, as does increased vascular permeability triggered by damage to the underlying connective tissue.

In experimentally infected, captive *M. lucifugus*, WNS disrupts several other physiological processes during hibernation, leading to hypovolemia, hypotension, and reduced capillary refill, causing local hypoxia and anaerobic lactic acid production; electrolyte depletion (decreased plasma sodium and chloride levels); metabolic acidosis due to lower carbon dioxide partial pressure and higher lactic acid levels; increased hematocrit; and hypoglycemia. Many of these alterations have their roots in the loss of fluids and nutrients via depletion of fat stores (Cryan *et al.* 2013; Warnecke *et al.* 2013).

13.2.2 The role of torpor in WNS disease dynamics

Normal hibernation is characterized by bouts of torpor interrupted by brief periods of arousals to normothermic body temperature. Arousals typically occur an average of every 2–3 weeks and last several hours (Jonasson & Willis 2012). Deep torpor during hibernation depresses all physiological processes, including immunological activity, a vital component of energy conservation in which the costs and benefits of arousals are balanced with those of torpor. Depth and length of bouts of torpor, frequency of periodic arousals, and minimum body temperatures may vary greatly among species of hibernating bats. Temperature radio-telemetry also revealed large variations in the duration of torpor bouts among individual, uninfected *M. lucifugus* at 4 °C in a cave in central Manitoba, Canada. This intraspecies variation did not correlate with age, sex, or body condition. The lack of correlation between sexes, however, may be due to low sample size. Overall, *M. lucifugus* utilized a more energetically conservative hibernation pattern than that seen in many species of rodents and might be due to spending a greater proportion of time in deep torpor and utilizing shallow torpor bouts during arousal (Jonasson & Willis 2012). The use of shallow torpor might serve as a unique adaptation to allow energy conservation during the costliest phase of hibernation.

The cave-roosting members of *C. rafinesquii* in the northern portions of their range do not display signs of WNS even though *P. destructans* has been found on some individuals of this species. Radio-tagged *C. rafinesquii* from Mammoth Cave were found to have short (average of 2.4 days) and shallow torpor bouts and changed roosts approximately every 4 days, some traveling almost 6 km between consecutive roosts during the winter. This pattern is more like that seen in European than in North American bats. The probability of arousal in *C. rafinesquii* was linked with higher ambient temperature at sunset, when 83% ($n = 86$) of the arousals occurred, especially on warm nights. *C. rafinesquii* are shallow hibernators that are relatively active during winter, including participating in mating. This hibernation pattern may function as a defense mechanism against WNS pathology possibly due to increased immune system activity during arousal (Johnson *et al.* 2012). Uninfected *M. lucifugus* had average torpor bouts of 16.3 days and even bats that died from WNS had average torpor bout durations over two times greater than that recorded for *C. rafinesquii*. The European bat, *P. auritus*, however, frequently arouses during hibernation and has been found to have deep *P. destructans*-induced lesions.

Physiological (timing of arousal, rewarming rate) and behavioral (arousal synchronization, clustering) were studied for 4 months in experimentally infected *M. lucifugus* in order to test two competing hypotheses: (1) bats synchronize arousal with other bats

to compensate for increased energy-expensive arousals by thermoregulation; or (2) changes in arousal physiology and clustering are maladaptive consequences of infection. The latter hypothesis predicts that disturbance by normothermic bats contributes to increased arousal frequency. Turner *et al*. (2015) found that while arousals of infected bats is more synchronized than seen in uninfected animals as hibernation progressed, the pattern was not consistent with social thermoregulation. Bat rewarming from torpor often triggered an arousal cascade of up to seven other bats, rather than simultaneously, as would be expected for energy conservation due to social thermoregulation. Furthermore, the rewarming rate of infected bats was similar to that observed in uninfected animals and did not change over time. It has also been suggested that passive rewarming rates in synchronized arousals in clustered bats would be slower than that seen in individual bats since passive rewarming is slower than active rewarming rates. This study, however, found no significant differences in rewarming rates of clustered bats versus bats roosting alone or in warming rates for cascading arousals versus that occurring in isolated animals. Altered vocalizations of the bats may also trigger disturbance in other hibernating animals, although this was not addressed in the study. Taken together, these findings suggest that maladaptive disturbances and not social thermoregulation explain the increased synchronization of arousals (Turner *et al.* 2015). These results also suggest that the density of bats in an infected hibernaculum would correlate with increased arousal frequency and mortality (Turner *et al.* 2015).

The adaptive purpose of arousals is not well known but it is hypothesized that more frequent arousals might allow increased grooming activity to remove the fungus or permit drinking to counteract their state of dehydration. In an attempt to explore the validity of these hypotheses, captive *M. lucifugus* were experimentally infected with *P. destructans* and their activity observed from infrared video recordings. Infected animals tended to be less active than uninfected bats during arousal. Reduced activity may represent an energy-saving response or be a pathological consequence of infection. Grooming frequency was not increased nor was the number of visits to their water source.

Wilcox *et al*. (2014) also found that clustering behavior in the infected captive animals decreased progressively over the course of infection. *M. lucifugus* and *M. sodalis* typically cluster during hibernation (Clawson *et al.* 1980; Brack & Twente 1985). After the introduction of WNS, an increased proportion of bats roost individually, suggesting that WNS is either inducing a behavioral change or that it may select for bats that roost individually (Langwig *et al.* 2012). Reduced clustering may have consequences for transmission, as is the case for infected members of some colonial insect species which isolate, presumably to reduce transmission to relatives (reviewed by Wilcox *et al.* 2014). Clustering may reduce evaporative water loss and, therefore, bats roosting individually may become more dehydrated (Thomas & Cloutier 1992). Solitary roosting may, conversely, have survival benefits if it slows fungal growth by altering the microclimate to which the fungus is exposed (Wilcox *et al.* 2014). Reduced clustering behavior has also been shown to occur in *P. destructans*-infected *M. lucifugus* in a natural setting (Langwig *et al.* 2012).

13.2.3 WNS and wing damage

Infection of cutaneous glands may reduce wing waterproofing and allow water to be wicked from the wing and may reduce cutaneous respiration and, thus, passive gas exchange during torpor (Cryan *et al.* 2010). Disruption of passive gas-exchange pathways

has been suggested to lead to a compensatory increase in the more water-intensive pulmonary respiration, resulting in higher total evaporative water loss and dehydration. However, blocking passive gas-exchange pathways was found to not affect water loss, suggesting that another factor(s) leads to dehydration (Carey & Boyles 2015).

Since *P. destructans* invades sebaceous glands of the epidermis of bat wings, it may alter the epidermal polar lipid composition. Polar lipids were obtained from wing samples from three damaged and three healthy *M. lucifugus*. Lower levels of the following lipids were detected in damaged wing tissue: ether-linked phospholipids, lysophospholipids, phosphatidylcholine, and phosphatidylethanolamine (Pannkuk *et al.* 2015a). Six unsaturated glycerophospholipids were not present in the damaged tissue. Altered lipid composition negatively impacts several physiological functions, including the innate immune system activity and water retention.

Fungi secrete proteases and other enzymes to digest complex environmental substrates for nutrition. Extracellular proteases break peptide bonds in proteins to yield amino acids for assimilation. Secreted serine proteases have been implicated in fungal pathogenesis. Pannkuk *et al*. (2015b) isolated a major secreted 27.9 kDa *P. destructans* protein and determined it to be a S8A subtilisin-like serine protease (PdSP1). This protease may play an important role in destruction of bat wing tissue, but further *in vitro* and *in vivo* studies are required in order to clarify its role in *P. destructans* pathogenesis.

13.3 THE GEOGRAPHICAL DISTRIBUTION OF WHITE-NOSE SYNDROME

13.3.1 WNS in North America

P. destructans may have been introduced into North America from Europe. This is supported by the finding that isolates of this fungus from various sites in the eastern US appear to be genetically identical and may have been derived from a single introduction (Ren *et al.* 2012). The sequences are distinct from the closely related *Geomyces pannorum* that, on rare occasions, causes human skin and nail infections (Chibucos *et al.* 2013).

WNS was first found in North America in Howes Cave in New York state during the winter of 2006–2007 and is spreading south and westward (Foley *et al.* 2011). In order to determine the rapidity of the infection's spread, five bat species from two hibernacula in Central Illinois were studied over time. During the winter of 2012–2013, only one *Myotis septentrionalis* was PCR-positive ($n = 129$) and no fungi were detected on the hibernaculum substrates. In the following March, however, greater than 85% of tested *M. septentrionalis* and *M. lucifugus* were infected, as well as 40–75% of *Eptesicus fuscus* and *M. sodalist*, and 15–60% of *Perimyotis subflavus*. By November, 98% of the bats were infected and an extensive fungal presence was found throughout the hibernacula (Langwig *et al.* 2015b). *P. destructans* is found in 29 states and 5 provinces in the US and Canada, respectively, and the fungus is known to be present in three additional states. The WNS has already killed roughly 6 million bats living in the US and Canada (Bat Conservation International 2017). Twenty-five species of insectivorous bats hibernate in the US and Canada and are therefore at risk of developing disease (U.S. Geological Survey 2012). *P. destructans* was also detected in a moribund *M. lucifugus* in the Pacific northwestern US and was indistinguishable from the clonal *P. destructans* populations in the Eastern US and Canada (Lorch *et al.* 2016).

The following bat species are infected by *P. destructans* in North America and subsequently developed WNS: the big brown bat (*Eptesicus fuscus*), the Eastern small-footed myotis (*Myotis leibii*), the little brown bat (*M. lucifugus*), the northern long-eared bat (*M. septentrionalis*), the Indiana bat (*M. sodalis*), the tricolored bat (*Pipistrellus subflavus*), and the gray bat (*Myotis grisescens*). *P. destructans* has been found in the following species of bats that have not developed WNS: the southeastern bat (*Myotis austroriparius*), the silver-haired bat (*Lasionycteris noctivagans*), the Virginia big-eared bat (*Corynorhinus townsendii virginianus*), the Eastern red bat (*Lasiurus borealis*), and Rafinesque's big-eared bat (*Corynorhinus rabinesquii*). Of these species, *M. grisescens* and *M. sodalis* are endangered and *M. septentrionalis* is threatened (Bat Conservation International 2017). The solitary hibernators, *E. fuscus* and *M. leibii*, were least impacted by the disease (Langwig *et al.* 2012).

Alves *et al*. (2014) used mapping to predict potential regions into which *P. destructans* would spread in North America based on overlying the distribution of hibernating bats with environmental conditions currently present in infected sites. Their results indicate that WNS will be primarily found in the east and southeast of the US, but could threaten 32% of the bat species.

At least one other fungal species is able to infect bat skin. Bats with superficial fungal skin infections were reported in bat carcasses in Wisconsin, Indiana, and Texas in 2011 and 2012. The affected skin regions appeared similar to those seen in WNS. Genetic sequencing implicates these fungal isolates as a new member of the *Trichophyton* genera that was named *Trichophyton redellii* (Lorch *et al.* 2015).

13.3.2 WNS in Europe

P. destructans has also been reported in 15 European countries (Austria, Belgium, Switzerland, the Czech Republic, Germany, Denmark, Estonia, France, Hungary, the Netherlands, Poland, Romania, Slovakia, Turkey, and Ukraine) and Asia, but has not caused the massive number of bat deaths or population changes as is seen in northern North America (reviewed by Kokurewicz *et al.* 2016). Lesions similar to those of North American WNS, however, were found in *P. destructans*-positive bats collected as they were emerging from their hibernacula in early spring of 2013 in the Czech Republic. These lesions were characterized by edema of connective tissue and derangement of fibroblasts and elastic fibers. Fungal invasion was associated with inflammatory infiltration of neutrophils between the infected and noninfected skin areas. The most frequent type of lesions were cup-like erosions packed with *P. destructans* hyphae invading the dermis. Invasive infection of the full wing membrane thickness was seen in specimens of *M. daubentonii* and *P. auritus* (Bandouchova *et al.* 2015). Nevertheless, European and North American strains of *P. destructans* are equally pathogenic for hibernating *M. lucifugus* in terms of the presence of hyphae, edema, necrosis, bacterial infection, local neutrophil infiltration, and inflammation (Warnecke *et al.* 2012, 2013). Inoculation of North American (NAPd) and European strains of *P. destructans* (EUPd) into *M. lucifugus* decreased the duration of torpor from an average of 16 days in uninfected control animals to 9 days and 6 days for NAPd and EUPd infected bats, respectively. Both NAPd and EUPd caused progressive increases in the frequency of periodic arousals by 3–4 times that of control animals by the conclusion of the study (Warnecke *et al.* 2012). Length of arousal was not affected. Both NAPd and EUPd caused white growth, loss of

elasticity, irregular pigmentation, stickiness of wing tissues, and fungal penetration of the epidermis, resulting in damage to underlying skin tissue (Warnecke *et al.* 2012). Interestingly, bats infected with NAPd survived longer than those infected with EUPd.

P. destructans has been detected by microscopic identification of *P. destructans* conidia, fungal culture, and genetic analysis in the following European bat species: *Myotis myotis, Myotis blythii, Myotis mystacinus, Myotis daubentonii, Myotis dasycneme, Myotis nattereri, Myotis bechsteinii, Myotis brandtii, Myotis alcathoe, Myotis velifer*, and *Myotis emarginatus, Eptesicus nilssonii, Rhinolophus hipposideros, Barbastella barbastellus*, and *Plecotus auritus* (Zukal *et al.* 2014; Kokurewicz *et al.* 2016). Since infected bat species are ecologically diverse and use a wide variety of hibernating strategies, *P. destructans* may be a generalist and may thus endanger any bats which hibernate within its geographical range (Zukal *et al.* 2014).

The microclimatic conditions in *P. destructans*-containing areas of an underground bat reserve in Poland were as follows: median temperature of 8.7 °C with minimum–maximum of 6.1–9.9 °C and humidity of 100% with minimum–maximum of 77.5–100.0%. These conditions were also preferred by hibernating *M. myotis* and *M. daubentonii*, suggesting that these species may be especially prone to infection (Kokurewicz *et al.* 2016).

13.3.3 WNS in Eastern Asia

Bats and hibernacula walls and ceilings from 12 sites and 3 provinces across northeastern China were swabbed during the spring or summer of 2014 and 2015. *P. destructans* DNA was detected from cave surfaces from 75% of tested sites ($n = 12$) and on the bats from 22% of the sites ($n = 9$) (Hoyt *et al.* 2016). While environmental *P. destructans* prevalence was low during the summer, the fungus was detected in 10% of tested *Myotis macrodactylus* ($n = 10$), 100% of *Myotis chinensis* ($n = 1$), and 100% of *Murina ussuriensis* ($n = 1$). In winter, prevalence was higher in the three species tested: 94.1% of *Myotis petax* ($n = 17$), 57.9% of *Rhinolophus ferrumequinum* ($n = 19$), and 68.8% of *Myotis leucogaster* ($n = 16$).

13.4 THE EFFECTS OF WHITE-NOSE SYNDROME ON SELECTED NORTH AMERICAN BAT POPULATIONS

13.4.1 WNS and *Myotis lucifugus*

The little brown bat (*M. lucifugus*) was once the most common hibernating bat in northeastern US, but may become extinct in this part of the country by 2024 since a 91% mortality rate is seen in their affected hibernacula. *M. lucifugus* has been divided into five morphological subspecies (*M. l. alascensis, M. l. carissima, M. l. lucifugus, M. l. pernox,* and *M. l. relictus*), but little is known about the amount of their genetic divergence. Based upon coalescent analyses of nuclear and mitochondrial DNA, some of these subspecies may represent independent evolutionary lineages (reviewed by Vonhof *et al.* 2015). It is important to determine whether the subspecies have different WNS-associated mortality rates.

Burns *et al*. (2014) studied the population genetic structure of *M. lucifugus* at swarming sites in southeastern Canada, many of which were in Nova Scotia. Analysis

of nuclear microsatellite and mitochondrial DNA from various swarming sites suggested high contemporary gene flow and thus a high degree of connectivity. They concluded that the mainland areas of the southeastern Canada may be considered to serve as one large gene pool for *M. lucifugus* (Burns *et al.* 2014). These findings may partially explain the rapid spread of WNS throughout the region. A study in the western portion of the range also found little variation among *M. lucifugus* summering areas, indicating high gene flow among subspecies in this region (Lausen *et al.* 2008).

In order to address the risk of WNS spreading from eastern populations to other populations of *M. lucifugus* in the US, Vonhof *et al.* (2015) studied the extent of genetic variation and population differentiation across the entire range of this bat species as well as the presence of barriers to gene flow that could block the geographic spread of WNS. Such studies are particularly important since the geographic range of *M. lucifugus* extends across the temperate regions of North America, possibly enabling transmission of *P. destructans* to species of North American hibernating bats that otherwise may be geographically isolated from the fungus. During the autumn, individual bats may migrate hundreds of kilometers between their summer and winter/autumn sites. Banding data from central Canada indicates extensive movements by individual bats of both sexes between summer roosts, swarming sites, and hibernacula (Norquay *et al.* 2013).

No barriers to gene flow were found over the range of *M. lucifugus*, but large amounts of spatial variation were observed in patterns of female dispersal and genetic variation between populations in the eastern and western populations. While nuclear genetic differentiation was low, mitochondrial DNA differentiation was highly variable among all western samples, between western and eastern samples, and among some eastern samples, but low mitochondrial differentiation was detected within two groups of samples from central and eastern regions of North America. The amount of nuclear DNA gene flow was less in western sites than those in the east. Populations in western, but not eastern, sites appear to be isolated by distance, perhaps in part, due to the greater topographical and ecological heterogeneity. Large hibernacula are only found in the eastern part of the *M. lucifugus* range. The high density of mines and caves in part of the western range contribute to the smaller and more diffuse hibernating colonies in western areas. Taken together, these findings suggest that the pattern of spread of WNS in eastern North America may differ in other areas of *M. lucifugus*' range and that the risk of WNS transmission and occurrence may vary if the disease continues to spread westward (Vonhof *et al.* 2015).

13.4.2 WNS and *Myotis sodalis*

The endangered Indiana bat (*M. sodalis*) is in danger of being locally and regionally extirpated. The model developed by Thogmartin *et al*. (2013) predicts that numbers of this bat species will reach their lowest point by 2020, but they will persist, in greatly reduced numbers, for at least 50 years. The ability of *M. sodalis* to survive extinction is predicated upon whether surviving populations can grow and expand into depleted portions of its former range. Other factors, however, are pushing this species into extinction, including alteration of hibernacula, colony disturbance, pesticide use, loss of summer habitat due to deforestation, and wind farms. These additional factors must also be addressed, not only for *M. sodalis*, but for other bat species, whether or not currently threatened or endangered.

13.5 THE BAT IMMUNE RESPONSE TO WHITE-NOSE SYNDROME

An active immune response is energy-consuming and stimulation leads to increased basal metabolic rate in doves, sparrows, and mice, in addition to decreased body mass (reviewed by Moore *et al.* 2011). In order to conserve their limited energy reserves, rodents (hamsters and squirrels) in deep torpor have been reported to reduce the humoral component of the secondary immune responses as well as serum complement activities (reviewed by Moore *et al.* 2011). T and B lymphocyte proliferation and numbers are reduced and the lymphocytes are sequenced into secondary lymphoid organs. Neutrophils and macrophages are absent from *P. destructans*-infected sites in hibernating bats (Meteyer *et al.* 2012). In this section, various aspects of the immune response of hibernating versus active bats exposed to *P. destructans* will be examined.

13.5.1 Leukocyte counts

Various immune parameters were compared in *M. lucifugus* from confirmed WNS-affected and unaffected sites (Moore *et al.* 2013). No difference was seen between the groups in total circulating antibody levels, but leukocyte counts were higher in bats from affected sites, especially in bats with elevated body temperatures (above 20 °C). Leukocyte counts were not correlated with hematocrit, body mass index, or hibernation state.

13.5.2 Antifungal activity in the plasma

Plasma bactericidal and antifungal activity was compared between *M. lucifugus* at mid-hibernation at sites in which WNS is present with WNS-free sites. The former had higher bactericidal ability against *Escherichia coli* and *Staphylococcus aureus*, but lower activity against the fungus *C. albicans*. There were no differences in either activity, however, between bats with or without fungal infections (Moore *et al.* 2011).

13.5.3 T helper cell activity in infected bats

Bats from affected sites, especially those with visible fungal infections, had lower amounts of antioxidant activity and IL-4, a cytokine that induces T cell differentiation along the Th2 pathway that antagonizes the antifungal activity of the Th1 immune pathway. The changes in immune parameters could be a response to fungal infection or to altered thermoregulatory behaviors aroused from torpor (Moore *et al.* 2013). Decreased IL-4 levels in the infected bats may reflect an attempt to direct the immune away from the Th2 response and towards a Th1 response that is better suited to protect against fungi. Increasing energetically expensive immune functions may further drain infected bats' energy reserves and decreased antioxidant activity, in addition to the increased production of toxic reactive oxygen species resulting from greater amounts of aerobic respiration, may induce oxidative damage to tissue proteins, lipids, and nucleic acids.

13.5.4 Inflammatory activity in infected bats

Infected hibernating *M. lucifugus* had higher levels of RNA for the anti-inflammatory cytokine interleukin (IL)-10, the proinflammatory cytokines IL-23 and tumor necrosis factor-α, and the antibacterial and antifungal compound cathelicidin in their lungs than noninfected

bats, suggesting the possibility of a systemic immune response to *P. destructans* (Rapin *et al.* 2014). All of these molecules are produced by neutrophils or macrophages of the innate immune system. The levels of these molecules differed greatly and may correlate with the extent of pathology seen in infected bats. Levels of IL-10 correlated with level of *P. destructans* infection and number of hyphae and bacterial present in the wing, tumor necrosis factor-α correlated with *P. destructans* levels and number of wing hyphae, while IL-23 and cathlicidin levels correlated with neutrophil accumulation and inflammation. It is unlikely that transcription occurs during the severely reduced metabolic state present in hibernation, so these genes may be transcribed during arousal periods (Rapin *et al.* 2014).

A separate study of changes in the transcriptosome of wing cells of *P. destructans*-infected hibernating bats versus uninfected bats revealed that a local acute inflammatory response did occur, with increased expression of genes involved in inflammation, wound healing, and metabolism (Field *et al.* 2015). More inflammatory cytokine transcripts were produced, including IL-1β, IL-6, IL-17C, IL-20, IL-23A, IL-24, and G-CSF. The levels of the Ccl2 and Ccl20 chemokines were also increased, however, without accumulation of neutrophils and T lymphocytes in the affected area. Expression of acute inflammatory genes was also increased, including cyclooxygenase-2, which triggers production of eicosanoids as well as the nociception mediators kallikrein-6 and cathepsin S. This may lead to pain and itching and contribute to increased arousals. These immune mediators may be produced by local keratinocytes and fibroblasts to support wound healing. RNA levels for the transcription factors p65 and NFκB and the P-selectin glycoprotein ligand 1 were also increased as were CD3γ and CD45 levels, perhaps indicative of the presence of skin gamma delta T cells. This local inflammatory response may act as a double-edged sword, protecting the hibernating bats against the fungal infection, but may contribute to morbidity and mortality by affecting torpor behavior or inducing damage after emergence from hibernation (Field *et al.* 2015). This study also found that expression of apolipoproteins, lipid transport proteins, and protein and carbohydrate metabolism genes were up-regulated, including hydroxycarboxylic acid receptors 2 and 3 which mediate adiponectin secretion. The resulting changes in lipid and carbohydrate metabolism may contribute to depletion of fat stores (Field *et al.* 2015). The observed up-regulation of oxidative stress genes may also lead to local tissue damage.

13.5.5 Differences in the immune response to WNS in European and North American bats

P. destructans-infected hibernating European bats are not experiencing the massive population losses found in some North American bat populations. The difference might lie in differing aspects of the immune response in these two populations. Johnson *et al.* (2015) explored possible difference in antibodies. Interestingly, seroprevalence and titers of anti-*P. destructans* antibodies are greater among experimentally infected members of *M. lucifugus* than in four other species of North American cave-hibernating bats (*P. subflavus, E. fuscus, M. septentrionalis*, and *Corynorhinus rafinesquii*), some of which have much lower WNS mortality rates. The highest antibody titers were found in the spring and in naturally infected *M. lucifugus* populations in regions where WNS has been known to occur for longer time periods. Cross-reactive antibodies were also detected in bats with no prior history of *P. destructans* infection. By contrast, no *P. destructans*-specific antibodies were found in naturally infected *M. myotis* and *M. daubentonii*

European bats during the winter hibernation and titers were lower than *M. lucifugus* during other times of the year (Johnson *et al.* 2015). Taken together, antibody responses against *P. destructans* do not appear to prevent or mitigate WNS but may instead lead to a state of tolerance that allows chronic infection (Casadevall & Pirofski 2012). Cell-mediated immunity may, however, play the major role in protecting European bats against fungal-induced pathology, as is the case in mice infected with *Candida* (Spellberg *et al.* 2008). This hypothesis needs to be tested in order to measure the extent of the protective immunity as well as the cells and mechanisms involved.

13.5.6 Immune-mediated pathology in WNS

Despite the suppression of immune responses occurring during hibernation, within weeks after *M. lucifugus* returned to an active state, an intense neutrophilic inflammatory response is produced against *P. destructans* that may cause severe pathology or death in the bat host. About 3 weeks after arousal, progressive damage begins in the wing. The bats become moribund and unable to fly. This inflammatory reaction continues for another 3 weeks before visible signs of healing begin (Meteyer *et al.* 2012). Sudden reversal of immune suppression in bats appears to lead to a form of immune reconstitution inflammatory syndrome, first reported in HIV-positive people, which results in rapid worsening of symptoms after restoration of immunity during an ongoing infection. In bats, the rapid influx and degranulation of neutrophils in the sites of fungal infection leads to edema and necrosis and sequestration of fungal hyphae into networks of degenerative cell material. The intensity and extent of fungal infection is a determining factor in whether the intense inflammatory response produced upon emergence from hibernation will lead to severe tissue damage and death or will eliminate the fungi, resulting in recovery (Meteyer *et al.* 2012).

13.6 ANTIFUNGAL AGENTS

13.6.1 Antifungal compounds

High-throughput screening of the SpectrumPlus compound library found several agents to which *P. destructans* was susceptible at concentrations within the range used to treat pathogenic fungi of humans. *P. destructans* was susceptible to amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole. Of the 1920 compounds in the library, 27 inhibited growth by 50–90% at 15 °C, an appropriate temperature for use in underground environments (Chaturvedi *et al.* 2011). The methods of treatment need to be carefully studied, since in the case of amphibians suffering from chytridiomycosis, application of itraconazole to tadpoles was effective against *B. dendrobatidis* fungi, but caused depigmentation in the amphibian hosts (Garner *et al.* 2009). Use of these agents to decontaminate hibernacula also requires caution and they need to be tested for long-term effects and toxicity to other cave or mine inhabitants (Chaturvedi *et al.* 2011).

A single application of cold-pressed, terpeneless orange oil (10 µl of 100% oil) completely inhibited *P. destructans* growth *in vitro* cultures for at least 6months of incubation at both 4 °C and 15 °C (Boire *et al.* 2016). This oil, at a concentration of 100%, did not affect growth of other environmental microorganisms, including various filamentous fungi, bacteria, and aerobic actinomycetes. It is used as a flavoring agent in

foods, in cosmetics, and in cleaning products and is not toxic to mammalian keratinocytes. Further testing, however, is required to determine the specific effects of this oil on bats, particularly on their wing structures. Test designs should also take into account whether the oil is removed by the bats during grooming or affected by high body temperatures occurring during flight, if it is applied to active animals.

13.6.2 Antifungal agents derived from bacteria

The skin microbiota of different species of bats or even individuals within a given species may play a role in the severity of skin diseases, including WNS. Beneficial bacteria (probiotics) of the skin microbiome with antifungal activity may be introduced onto bat skin and might be able to coevolve within skin pathogens, providing long-lasting protection (Thomas & Willis 1998). This strategy of bioaugmentation of probiotics isolated from the skin of a host species was effective in a field trial to protect amphibians from chytridiomycosis. Probiotics from the skin of a resistant animal species may be able to colonize and protect another, similar susceptible host species (Bletz *et al.* 2013). Isolates of the bacterial genera *Pseudomonas* isolated from bat skin inhibited growth of *P. destructans in vitro* for at least 35 days. Growth suppression was measured in a laboratory setting by the formation of a zone of inhibition in a fungal lawn around the tested bacterial isolates and was found to be dependent on initial *P. destructans* and bacteria concentrations. The isolates used in this study appear to belong to the *Pseudomonas fluorescens* group, previously shown to produce compounds that inhibit growth of fungal pathogens of plants and amphibians (reviewed by Hoyt *et al.* 2015a). This work is encouraging, but must be tested for toxicity and efficacy *in vivo*. Even if *P. destructans* were not eliminated from an infected bat, slower growth patterns may allow the bat to survive the winter (Hoyt *et al.* 2015a).

At least two-thirds of commercial antibiotics have been derived from the *Streptomyces* genus of Actinobacteria, making these cave-dwelling-bacteria potential sources of compounds which inhibit *P. destructans* growth. Thirty-six Actinobacteria, 88.9% of them *Streptomyces*, isolated from WNS-free bats in the southwestern US were able to stop or slow growth of *P. destructans* (Hamm *et al.* 2016). Another bacterium, *Rhodococcus rhodochrous*, was shown to completely and permanently block *P. destructans* spore germination, in addition to slowing growth of the hyphae (Cornelison *et al.* 2014b).

Volatile organic compounds produced by *Pseudomonas* and *Bacillus* bacterial species in soil inhibited *P. destructans* growth from conidia and radial mycelial extension. Inhibitory activity was greater at 4 °C than at 15 °C. Decanal, 2-ethyl-1-hexanol, nonanal, benzothiazole, benzaldehyde, and *N*,*N*-dimethyloctylamine were all inhibitory to *P. destructans* growth at concentrations of less than 1 part per million. Several combinations of these compounds had synergistic activity against *P. destructans*. Some such volatile organic compounds are now being used to eliminate odors and control pests (Cornelison *et al.* 2014a).

13.6.3 Antifungal agents derived from fungi

Trichoderma polysporum fungi isolated from an air sample from a cave in a *P. destructans*-affected region caused a four log reduction in *P. destructans* colony-forming units (Zhang *et al.* 2015). The fungi had specific fungicidal activity against *P. destructans* but

not against *P. pannorum* and retained this activity even after exposure to high temperatures and light. Since these microbes were isolated from a cave, they are highly adapted to growth at temperatures of 6–15 °C. *Trichoderma* species are known to have biocontrol properties and have been approved for commercial use for the biocontrol of agriculture pests in the US. *T. polysporum* produces several secondary metabolites with antifungal activity, including trichosporin, cyclosporine, peptaibols, and cyclonerodiol derivatives. Further work is needed in order to determine which of these compounds or combination of compounds has activity against *P. destructans* (Zhang *et al.* 2015).

Sesquiterpene *trans*, *trans*-farnesol, produced by the yeast *Candida albicans*, blocked *P. destructans* conidial germination *in vitro* for at least 2 weeks and inhibited growth of preexisting hyphae at 10 °C in concentrations as low as 15–20 μM, which is within the range excreted by some *Candida* isolates. Additionally, *P. destructans* is more sensitive to the inhibitory effects than other North American *Pseudogymnoascus* (Raudabaugh & Miller 2015). This compound caused negligible toxic effects in mice and is inactivated upon prolonged exposure to oxygen, thus limiting any potential adverse environmental impact.

13.7 THE MYCOBIOME OF WHITE-NOSE SYNDROME-INFECTED HIBERNACULA

Lorch *et al.* (2013a) cultured a large number of fungi from the soil of 24 hibernacula in the eastern US during the winter of 2008–2009. The largest number of isolates were from the phylum Ascomycota, encompassing multiple orders and genera, as follows: 1–2 isolates each of *Pseudocercosporella, Arthrographis, Epicoccum, Dictyosporium, Arthroderma, Auxarthron, Gymnascella, Gymnoascoideus, Neogymnomyces, Mycoarthris, Candida, Debaryomyces, Hypomyces, Neonectria*, and *Tolypocladium* and multiple isolates of *Geomyces, Aspergillus, Penicillium, Gymnoascus, Trichophyton, Oidiodendron, Pseudeurotium, Isaria, Nectria, Verticillium, Doratomyces, Kernia,* and *Trichocladium*. Helotiales was the most dominant order and *Geomyces* was the most dominant genus. Lesser numbers of isolates were from the phylum Basidiomycota, encompassing three orders and three species: 1–2 isolates of *Coprinellus* and *Burgoa/Sistotrema* and multiple *Trichosporon* isolates. The phylum Zygomycota was also found in lesser numbers and was represented by two orders and three genera containing multiple isolates of *Mortierella, Helicostylum*, and *Mucor*. A number of other isolates were not identified into phyla. Some of the above fungal groups are known to produce compounds with antifungal or antibacterial properties and may thus be the source of compounds that will retard the growth of *P. destructans* (Lorch *et al.* 2013a).

A separate study of the mycobiome of two caves and four mines in New York and New Jersey in 2010 also revealed a wide range of fungal species. Samples were collected from the floor or ledges directly below the roosting bats and often included bat feces and decomposed bat remnants. Samples also included swabs of wall and ceiling surfaces and drill holes within several centimeters of the roosting animals (Zhang *et al.* 2014). This study used culture-dependent and culture-independent detection methods, with differing results since many isolates determined by culture-independent methods detect many fungi, such as fastidious species, that are not able to be cultured using current methods. Culture-dependent methods also tend to be biased towards more

rapidly growing species. Culture-independent methods, however, rely on the quality of DNA available for amplification and the universality of the primers. Additionally, primer sets are not equally efficient in amplifying DNA from ascomycetes, basidiomycetes and early diverging fungal lineages (EDFL) (Zhang *et al.* 2014). Culture-dependent methods detected Ascomycota, Basidiomycota, Zygomycota, and EDFL, with the majority of isolates (76%) belonging to Ascomycota. Culture-independent methods detected Ascomycota, Basidiomycota, Chytridiomycota, EDFL, Glomeromycota, and nonfungal species (Zhang *et al.* 2014). Use of different methodologies may allow the detection of a wider range of fungal phyla.

13.8 RECOVERY AND RECOLONIZATION

Since *P. destructans* is capable of persisting in the environment in the absence of their hosts, the local population of bats which survive the winter may be re-infected during the next hibernation period (Lorch *et al.* 2013b; Hoyt *et al.* 2015b). Long-term environmental persistence of *P. destructans* in the absence of bats has important implications for possible bat recolonization of hibernacula. Viable *P. destructans* was cultured in the laboratory for over 5 years from dried agar plates at 5 °C and low relative humidity (30–40%). These findings suggest that *P. destructans* can persist in the absence of bats for long periods, possibly preventing recolonization of hibernacula from which bat populations had been extirpated (Hoyt *et al.* 2015b). These findings imply that recolonization efforts may be more difficult than otherwise anticipated. This study also suggests that *P. destructans* may also be able to survive on equipment or clothing of cavers or other cave visitors, if stored in cool dry conditions, facilitating human-induced spread of the fungus (Reynolds & Barton 21014). This research needs to be expanded from the laboratory to known bat hibernation sites, which have higher relative humidity (>70%). Additionally, other microbiota (bacteria, viruses, protists, and other fungi) are present in hibernacula and may impact *P. destructans* survival *in situ* (Hoyt *et al.* 2015b).

Reynolds *et al*. (2015) modeled possible contributions of environmental *P. destructans* growth to WNS disease progression in *M. lucifugus*. Their model predicted that environmental growth of *P. destructans* would increase WNS infection rates, especially in hibernacula containing high levels of organic debris and would allow *P. destructans* to persist within infected hibernacula for decades, negatively impacting recolonization efforts.

Other examples of infectious disease agents that have devastated animal and plant populations include chytridiomycosis in amphibians, caused by the fungus *Batrachochytrium dendrobatidis*; West Nile virus in crows and jays in the New World (reviewed by Alves *et al.* 2014); *Varroa* mites in American honeybees (Sammataro *et al.* 2000); and American chestnut and American elm trees being affected by *Cryphonectria parasitica* and *Ophiostoma ulmi* fungi (Schlarbaum *et al.* 2017). Resistant tree species have been found and it is hoped may be successfully reintroduced into their former ranges. A potential problem lies in whether they will be able to reoccupy the niche they formerly held and successfully compete with the plants currently present.

Comparison of characteristics of a population of *E. fuscus* 4–7 years before the arrival of *P. destructans* with that found 2–3 years after introduction found differences in the length of the torpor bouts. Significantly, the mean body fat content of *E. fuscus* in late winter was almost double that of *M. lucifugus* hibernating at the same infected sites.

In one site, a population decline of 99.6% occurred in *M. lucifugus* after introduction of the fungus, while numbers of *E. fuscus* increased 43% during the same time period. While none of the *E. fuscus* had visible fungal growth or lesions, almost all *M. lucifugus* had growth on their wings, muzzle, and ears. These results suggest that at least this population of *E. fuscus* is resistant to WNS (Frank *et al.* 2014). In keeping with these findings, some European bat species confine *P. destructans* to the outer epidermis, blocking the deep invasion of hyphae, development of skin lesions, and frequent arousals from torpor (Wibbelt *et al.* 2013).

Some *M. lucifugus* colonies in New York appear to be persisting after the initial sharp population decline, with some colonies stabilizing at 5–30% of their original size (Puechmaille *et al.* 2011; Langwig *et al.* 2012; Hoyt *et al.* 2016). Langwig *et al*. (2017) offer a hopeful note by finding that the infection intensity of *P. destructans* was much lower in persisting than in declining populations recently invaded by the fungus. The authors' models are most consistent with fungal growth reduction, reducing bat morbidity and mortality. This is characteristic of resistance to infection rather than tolerance, which would permit persistent fungal infection. Asian bats have lower levels of *P. destructans* and thus resistance in these bat populations may be partially responsible for the lower mortality seen in these bats. Their models also indicate that the reduction in fungal growth is not due to density-dependent effects. Resistance may be due to changes in bat skin microbial communities, which may contain more bacterial or fungal species having anti-*P. destructans* activity, as discussed above. It may also be due to bat-reduced decrease in fungal food resources, activation of an immune response as a fungal load threshold is reached, or slow immune response activation by bats, as discussed above (reviewed by Langwig *et al.* 2017).

REFERENCES

Alves DMCC, Terribile LC, Brito D. 2014. The potential impact of white-nose syndrome on the conservation status of North American bats. *PLoS ONE.* 9(9):e107395.

Bandouchova H, Bartonicka T, Berkova H, Brichta J, Cerny J, Kovacova V, Kolarik M, Kollner B, Kulich P, Martínkóva N, Rehak Z, Turner GG, Zukal J, Pikula J. 2015. *Pseudogymnoascus destructans*: Evidence of virulent skin invasion for bats under natural conditions, Europe. *Transboundary and Emerging Diseases.* 62:1–5.

Bat Conservation International. 2017. http://www.batcon.org/. Accessed January 11, 2017.

Blehert DS. 2012. Fungal disease and the developing story of bat white-nose syndrome. *PLoS Pathogens.* 8(7):e1002779.

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JT, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ, Stone WB. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science.* 323:227.

Bletz MC, Loudon AH, Becker MH, Bell SC, Woodhams DC, Minbiole KP, Harris RN. 2013. Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. *Ecology Letters.* 16:807–820.

Boire N, Zhang S, Khuvis J, Lee R, Rivers J, Crandall P, Keel MK, Parrish N. 2016. Potent inhibition of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome in bats, by cold-pressed, terpeneless, valencia orange oil. *PLoS ONE.* 11(2):e0148473.

Brack JR, Twente JW. 1985. The duration of the period of hibernation of three species of vespertilionid bats. I. Field studies. *Canadian Journal of Zoology.* 63:2952–2954.

Burns LE, Frasier TR, Broders HG. 2014. Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). *Ecology and Evolution.* 4(21):4130–4149.

Carey CS, Boyles JG. 2015. Interruption to cutaneous gas exchange is not a likely mechanism of WNS-associated death in bats. *Journal of Experimental Biology.* 218(Pt 13):1986–1989.

Casadevall A, Pirofski L-A. 2012. Immunoglobulins in defense, pathogenesis, and therapy of fungal diseases. *Cell Host Microbe.* 11:447–456.

Chaturvedi S, Rajkumar SS, Li X, Hurteau GJ, Shtutman M, Chaturvedi V. 2011. Antifungal testing and high-throughput screening of compound library against *Geomyces destructans*, the etiologic agent of geomycosis (WNS) in bats. *PLoS ONE.* 6(3):e17032.

Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li X, Peck MK, Ren P, Bopp DJ, Wood B, Samsonoff WA, Butchkoski CM, Hicks AC, Stone WB, Rudd RJ, Chaturvedi S. 2010. Morphological and molecular characterizations of psychrophilic fungus *Geomyces destructans* from New York bats with white nose syndrome (WNS). *PLoS ONE.* 5(5):e10783.

Chibucos MC, Crabtree J, Nagarj S, Chaturvedi S, Chaturvedi V. 2013. Draft genome sequences of human pathogenic fungus *Geomyces pannorum Sensu Lato* and bat white nose syndrome pathogen *Geomyces* (*Pseudogymnoascus*) *destructans*. *Genome Announcements.* 1(6):e01045–13.

Clawson RL, LaVal RK, LaVal ML, Claire W. 1980. Clustering behavior of hibernating *Myotis sodalis* in Missouri. *Journal of Mammalogy.* 6:245e253.

Cornelison CT, Gabriel KT, Barlament C, Crow SA Jr. 2014a. Inhibition of *Pseudogymnoascus destructans* growth from conidia and mycelial extension by bacterially produced volatile organic compounds. *Mycopathologia.* 177:1–10.

Cornelison CT, Keel MK, Gabriel KT, Barlament CK, Tucker TA, Pierce GE, Crow SA. 2014b. A preliminary report on the contact-independent antagonism of *Pseudogymnoascus destructans* by *Rhodococcus rhodochrous* strain DAP96253. *BMC Microbiology.* 14:246.

Cryan PM, Meteyer CU, Blehert DS, Lorch JM, Reeder DM, Turner GG, Webb J, Behr M, Verant M, Russell RE, Castle KT. 2013. Electrolyte depletion in white-nose syndrome bats. *Journal of Wildlife Diseases.* 49:398–402.

Cryan PM, Meteyer CU, Boyles JG, Blehert DS. 2010: Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology.* 8:135.

Field KA, Johnson JS, Lilley TM, Reeder SM, Rogers EJ, Behr MJ, Reeder DM. 2015. The white-nose syndrome transcriptome: activation of antifungal host responses in wing tissue of hibernating little brown myotis. *PLoS Pathogens.* 11(10):e1005168.

Foley J, Clifford D, Castle K, Cryan P, Ostfeld RS. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. *Conservation Biology.* 25(2):223–231.

Frank CL, Michalski A, McDonough AA, Rahimian M, Rudd RJ, Herzog C. 2014. The resistance of a North American bat species (*Eptesicus fuscus*) to white-nose syndrome (WNS). *PLoS ONE.* 9(12):e113958.

Gargas A, Trest MT, Christensen M, Volk TJ, Bleher DS. 2009. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon.* 108:147–154.

Garner TWJ, Garcia G, Carroll B, Fisher MC. 2009. Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. *Diseases of Aquatic Organisms.* 83:257–260.

Grieneisen LE, Brownlee-Bouboulis SA, Johnson JS, Reeder DM. 2015. Sex and hibernaculum temperature predict survivorship in white-nose syndrome affected little brown myotis (*Myotis lucifugus*). *Royal Society Open Science.* 2:140470.

Hamm PS, Caimi NA, Northup DE, Valdez EW, Buecher DC, Dunlap CA, Labeda DP, Lueschow S, Porras-Alfaro A. 2016. Western bats as a reservoir of novel *Streptomyces* species with antifungal activity. *Applied Environmental Microbiology*. pii: AEM.03057-16.

Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF, Kilpatrick AM. 2015a. Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS ONE.* 10(4):e0121329.

Hoyt JR, Langwig KE, Okoniewski J, Frick WF, Stone WB, Kilpatrick AM. 2015b. Long-term persistence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, in the absence of bats. *EcoHealth.* 12:330–333.

Hoyt JR, Langwig KE, Sun K, Lu G, Parise KL, Jiang T, Frick WF, Foster JT, Feng J, Kilpatrick AM. 2016. Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions. *Proceedings of the Royal Society B.* 283:20152861.

Hoyt JR, Sun K, Parise KL, Lu G, Langwig KE, Jiang T, Yang S, Frick WF, Kilpatrick AM, Foster JT, Feng J. 2016. Widespread bat white-nose syndrome fungus, Northeastern China. *Emerging Infectious Diseases.* 22(1):140–142.

Johnson JS, Lacki MJ, Thomas SC, Grider JF. 2012. Frequent arousals from winter torpor in Rafinesque's big-eared bat (*Corynorhinus rafinesquii*). *PLoS ONE* 7(11):e49754.

Johnson JS, Reeder DM, Lilley TM, Czirják GA, Voigt CC, McMichael JW III, Meierhofer MB, Seery CW, Lumadue SS, Altmann AJ, Toro MO, Field KA. 2015. Antibodies to *Pseudogymnoascus destructans* are not sufficient for protection against white-nose syndrome. *Ecology and Evolution.* 5(11):2203–2214.

Johnson LJ, Miller AN, McCleery RA, McClanahan R, Kath JA, Lueschow S, Porras-Alfaro A. 2013. Psychrophilic and psychrotolerant fungi on bats and the presence of *Geomyces* spp. on bat wings prior to the arrival of white nose syndrome. *Applied Environmental Microbiology*. 79(18):5465–5471.

Jonasson KA, Willis CKR. 2012. Hibernation energetics of free-ranging little brown bats. *The Journal of Experimental Biology.* 215: 2141–2149.

Kokurewicz T, Ogórek R, Pusz W, Matkowski K. 2016. Bats increase the number of cultivable airborne fungi in the "Nietoperek" bat reserve in western Poland. *Microbial Ecology.* 72(1):36–48.

Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Kilpatrick AM. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters.* 15(9):1050–1057.

Langwig KE, Frick WF, Reynolds R, Parise KL, Drees KP, Hoyt JR, Cheng TL, Kunz TH, Foster JT, Kilpatrick AM. 2015a. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of the Royal Society B.* 282:20142335.

Langwig KE, Hoyt JR, Parise KL, Frick WF, Foster JT, Kilpatrick AM. 2017. Resistance in persisting bat populations after white-nose syndrome invasion. *Philosophical Transactions of the Royal Society B*. 372: 20160044.

Langwig KE, Hoyt JR, Parise KL, Kath J Kirk D, Frick WF, Foster JT, Kilpatrick AM. 2015b. Invasion dynamics of white-nose syndrome fungus, Midwestern United States, 2012–2014. *Emerging Infectious Diseases.* 21(6):1026–1026.

Lausen CL, Delisle I, Barclay RMR, Strobeck C. 2008. Beyond mtDNA: nuclear gene flow suggests taxonomic oversplitting in the little brown bat (*Myotis lucifugus*). *Canadian Journal of Zoology.* 86:700–713.

Lorch JM, Lindner DL, Gargas A, Muller LK, Minnis AM, Blehert DS. 2013a. A culture-based survey of fungi in soil from bat hibernacula in the eastern United States and its implications for detection of *Geomyces destructans*, the causal agent of bat white-nose syndrome. *Mycologia.* 105(2):237–252.

Lorch JM, Minnis AM, Meteyer CU, Redell JA, White JP, Kaarakka HM, Muller LK, Lindner DL, Verant ML, Shearn-Bochsler V, Blehert DS. 2015. The fungus *Trichophyton redellii* sp. Nov. causes skin infections that resemble white-nose syndrome of hibernating bats. *Journal of Wildlife Diseases.* 51(1):36–47.

Lorch JM, Muller LK, Russell RE, O'Connor M, Lindner DL, Blehert DS. 2013b. Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the eastern United States. *Applied Environmental Microbiology.* 79:1293–1301.

Lorch JM, Palmer JM, Lindner DL, Ballmann AE, George KG, Griffin K, Knowles S, Huckabee JR, Haman KH, Anderson CD, Becker PA, Buchanan JB, Foster JT, Blehert DS. 2016. First detection of bat white-nose syndrome in western North America. *mSphere*.1(4):e00148.

Lučan RK, Bandouchova H, Bartonička T, Pikula J, Zahradníková A Jr, Zukal J, Martínková N. 2016. Ectoparasites may serve as vectors for the white-nose syndrome fungus. *Parasites and Vectors.* 9:16.

Meteyer CU, Barber D, Mandl JN. 2012. Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence.* 3(7):583–588.

Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn-Bochsler V, Thomas NJ, Gargas A, Behr MJ. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation.* 21(4):411–414.

Moore MS, Reichard JD, Murtha TD, Nabhan ML, Pian RE, Ferreira JS, Kunz TH. 2013. Hibernating little brown myotis (*Myotis lucifugus*) show variable immunological responses to white-nose syndrome. *PLoS ONE.* 8(3):e58976.

Moore MS, Reichard JD, Murtha TD, Zahedi B, Fallier RM, Kunz TH. 2011. Specific alterations in complement protein activity of little brown myotis (*Myotis lucifugus*) hibernating in white-nose syndrome affected sites. *PLoS ONE.* 6(11):e27430.

Norquay KJO, Martinez-Nuñez F, Dubois JE, Monson KM, Willis CKR. 2013. Long-distance movements of little brown bats (*Myotis lucifugus*). *Journal of Mammalogy.* 94:506–515.

Pannkuk EL, McGuire LP, Warnecke L, Turner JM, Willis CK, Risch TS. 2015a. Glycerophospholipid profiles of bats with white-nose syndrome. *Physiological and Biochemical Zoology.* 88(4):425–432.

Pannkuk EL, Risch TS, Savary BJ. 2015b. Isolation and identification of an extracellular subtilisin-like serine protease secreted by the bat pathogen *Pseudogymnoascus destructans*. *PLoS ONE.* 10(3):e0120508.

Puechmaille SJ, Frick WF, Kunz TH, Racey PA, Voigt CC, Wibbelt G, Teeling EC. 2011. White-nose syndrome: is this emerging disease a threat to European bats? *Trends in Ecology and Evolution.* 26:570–576.

Rapin N, Johns K, Martin L, Warnecke L, Turner JM, Bollinger TK, Willis CKR, Voyles J, Misra V. 2014. Activation of innate immune-response genes in little brown bats (*Myotis lucifugus*) infected with the fungus *Pseudogymnoascus destructans*. *PLoS ONE.* 9(11):e112285.

Raudabaugh DB, Miller AN. 2015. Effect of trans, trans-farnesol on *Pseudogymnoascus destructans* and several closely related species. *Mycopathologia.* 180(5–6):325–332.

Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, Britzke ER, Vodzak ME, Darling SR, Stihler CW, Hicks AC, Jacob R, Grieneisen LE, Brownlee SA, Muller LK, Blehert DS. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE.* 7(6):e38920.

Ren P, Haman KH, Last LA, Rajkumar SS, Keel MK, Chaturvedi V. 2012. Clonal spread of *Geomyces destructans* among bats, Midwestern and Southern United States. *Emerging Infectious Diseases*. 18:883– 885.

Reynolds HT, Barton HA. 2014. White-Nose syndrome: Human activity in the emergence of an extirpating mycosis. In: *One Health: People, Animals, and the Environment*. RM Atlas and S Maloy (eds). ASM Press: Washington, DC, pp. 167–181.

Reynolds HT, Ingersoll T, Barton HA. 2015. Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the white-nose syndrome epidemic. *Journal of Wildlife Diseases.* 51(2):318–331.

Sammataro D, Gerson U, Needham G. 2000. Parasitic mites of honey bees: life history, implications, and impact. *Annual Review of Entomology*.45:519–548.

Schlarbaum SE, Hebard F, Spaine PC, Kamalay JC. 2017. Three American tragedies: chestnut blight, butternut canker, and Dutch elm disease. https://www.srs.fs.usda.gov/pubs/ja/ja_schlarbaum002.htm. Accessed January 11, 2017.

Spellberg B, Ibrahim AS, Lin L, Avanesian V, Fu Y, Lipke P, Otoo H, Ho T, Edwards JE Jr. 2008. Antibody titer threshold predicts anticandidal vaccine efficacy even though the mechanism of protection is induction of cell-mediated immunity. *Journal of Infectious Diseases.* 197:967–971.

Thogmartin WE, Sanders-Reed CA, Szymanski JA, McKann PC, Pruitt L, King RA, Runge MC, Russell RE. 2013. White-nose syndrome is likely to extirpate the endangered Indiana bat over large parts of its range. *Biological Conservation.* 160:162–172.

Thomas DW, Dorais M, Bergeron J-M. 1990. Winter energy budgets and cost of arousals for hibernating little brown bats *Myotis lucifugus*. *Journal of Mammalogy.* 71:475–479.

Thomas MB, Willis AJ. 1998. Biocontrol-risky but necessary? *Trends in Ecology and Evolution.* 13:325–329.

Thomas, WD, Cloutier, D. 1992. Evaporative water loss by hibernating little brown bats, *Myotis lucifugus. Physiological Zoology.* 65:443e456.

Turner JM, Warnecke L, Wilcox A, Baloun D, Bollinger TK, Misra V, Willis CKR. 2015. Conspecific disturbance contributes to altered hibernation patterns in bats with white-nose syndrome. *Physiology & Behavior.* 140:71–78.

U.S. Geological Survey. 2012. White-nose syndrome threatens the survival of hibernating bats in North America. https://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/. Accessed May 23, 2017.

Vanderwolf KJ, Malloch, McAlpine DF. 2016. Ectomycota associated with arthropods from bat hibernacula in eastern Canada, with particular reference to *Pseudogymnoascus destructans*. *Insects.* 7:16.

Verant ML, Boyles JG, Waldrep W Jr, Wibbelt G, Blehert DS. 2012. Temperature-Dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. *PLoS ONE.* 7(9):e46280.

Vonhof MJ, Russell AL, Miller-Butterworth CM. 2015. Range-wide genetic analysis of little brown bat (*Myotis lucifugus*) populations: estimating the risk of spread of white-nose syndrome. *PLoS ONE.* 10(7):e0128713.

Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, Wibbelt G, Blehert DS, Willis CKR. 2012. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Science USA*. 109:6999–7003.

Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G, Willis CKR. 2013. Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. *Biology Letters.* 9:20130177.

Wibbelt G, Puechmaille SJ, Ohlendorf B, Mühldorfer K, Bosch T, Görföl T, Passior K, Kurth A, Lacremans D, Forget F. 2013. Skin lesions in European hibernating bats associated with *Geomyces destructans*, the etiologic agent of white-nose syndrome. *PLoS ONE.* 8(9):e74105.

Wilcox A, Warnecke L, Turner JM, McGuire LP, Jameson JW, Misra V, Bollinger TC, Willis CKR. 2014. Behaviour of hibernating little brown bats experimentally inoculated with the pathogen that causes white-nose syndrome. *Animal Behaviour.* 88:157e164.

Willis CKR, Menzies AK, Boyles JG, Wojciechowsk MS. 2011. Evaporative water loss is a plausible explanation for mortality of bats from white-nose syndrome. *Integrative and Comparative Biology.* 51(3):364–373.

Zhang T, Chaturvedi V, Chaturvedi S. 2015. Novel *Trichoderma polysporum* strain for the biocontrol *of Pseudogymnoascus destructans*, the fungal etiologic agent of bat white nose syndrome. *PLoS ONE.*10(10):e0141316.

Zhang T, Victor TR, Rajkumar SS, Li X, Okoniewski JC, Hicks AC, Davis AD, Broussard K, LaDeau SL, Chaturvedi S, Chaturvedi V. 2014. Mycobiome of the bat white nose syndrome affected caves and mines reveals diversity of fungi and local adaptation by the fungal pathogen *Pseudogymnoascus (Geomyces) destructans*. *PLoS ONE.* 9(9):e108714.

Zukal J, Bandouchova H, Bartonicka T, Berkova1 H, Brack V, Brichta J, Dolinay M, Jaron KS, Kovacova V, Kovarik M, Martínková N, Ondracek H, Rehak Z, Turner GG, Pikula J. 2014. White-nose syndrome fungus: a generalist pathogen of hibernating bats. *PLoS ONE.* 9(5): e97224.

14

HISTOPLASMA CAPSULATUM AND OTHER FUNGI AND BATS

14.1 FUNGAL SPECIES AND BATS

14.1.1 *Histoplasma capsulatum*

Histoplasma capsulatum is a thermically dimorphic fungus, living as a soil saprophyte, especially in soil enriched with bird or bat guano, and converting to a yeast phase at 37 °C. The yeast is parasitic, pathogenic, and intracellular, while the mycelial phase serves as the infective form. Isolates have been divided into the *capsulatum, duboisii*, and *farciminosum* variants (Carter *et al.* 2001). The var. *H. duboisii* group is found only in tropical areas of Africa and causes cutaneous, subcutaneous, and bone lesions. The var. *H. farciminosum* isolates are present in Europe, Northern Africa, India and Southern Asia. They typically infect horses and mules (reviewed by Teixeira *et al.* 2016). Microsatellite analysis indicates that the *H. capsulatum sensu lato* complex contains at least eight clades with distinct geographical populations: Australia, Netherlands, Eurasia, North American class 1 and class 2 (NAm 1 and NAm 2), Latin American group A and group B (LAm A and LAm B) and Africa. Except for the Eurasian cluster, these clades are considered phylogenetic species. Teixeira *et al.* (2016) have proposed the addition of five new phylogenetic species that are nested within the LAm clades: LAm A1, LAm A2, LAm B1, LAm B2, RJ, and BAC-1. The authors proposed that bats may play a major role in *Histoplasma* speciation since monophyletic clades are associated with different species of bats. See Table 14.1 for a list of fungi associated with bats. Upon examination of nine species of infected bats in Latin America, one genetic cluster with a high degree of similarity was seen in Brazil and Argentina. The fungal sub-clusters from Mexico had a much

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TABLE 14.1 Fungi associated with bats

Bat family	Bat common name	Bat species	Fungus
Phyllostomidae	Fringed fruit-eating bat	*Artibeus fimbriatus*	*Candida parapsilosis*
Phyllostomidae	Hairy fruit-eating bat	*Artibeus hirsutus*	*Histoplasma capsulatum*
Phyllostomidae	Hairy fruit-eating bat	*Artibeus hirsutus*	*Pneumocystis*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Candida guilliermondii*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Candida lusitaniae*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Candida pelliculosa*
Phyllostomidae	Great fruit-eating bat	*Artibeus lituratus*	*Kluyveromyces* sp.
Phyllostomidae	Lesser Antillean fruit-eating bat	*Brachyphylla cavernarum*	*Histoplasma capsulatum*
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	*Candida albicans*
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	*Coccidioides posadasii*
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	*Cryptococcus neoformans*
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	*Histoplasma capsulatum*
Phyllostomidae	Short-tailed fruit bat	*Carollia perspicillata*	*Trichosporon pullulans*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Candida* sp.
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Coccidioides posadasii*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Histoplasma capsulatum*
Phyllostomidae	Common vampire bat	*Desmodus rotundus*	*Kluyveromyces* sp.
Vespertilionidae	Big brown bat	*Eptesicus fuscus*	*Histoplasma capsulatum*
Molossidae	Dwarf bonneted bat	*Eumops bonariensis*	*Histoplasma capsulatum*
Molossidae	Wagner's bonneted bat	*Eumops glaucinus*	*Histoplasma capsulatum*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Blastomyces dermatitidis*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Candida albicans*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Candida guilliermondii*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Coccidioides posadasii*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Histoplasma capsulatum*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Pneumocystis*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Torulopsis colliculosa*
Phyllostomidae	Pallas's long-tongued bat	*Glossophaga soricina*	*Trichosporon pullulans*
Emballonuridae	Hairy slit-faced bat	*Nycteris hispida*	*Histoplasma capsulatum*
Phyllostomidae	Lesser short-nosed bat	*Leptonycteris curasoae*	*Histoplasma capsulatum*
Phyllostomidae	Mexican long-nosed bat	*Leptonycteris nivalis*	*Histoplasma capsulatum*

Phyllostomidae	Lesser long-nosed bat	*Leptonycteris sanborni*	*Cryptococcus albidus*
Phyllostomidae	Lesser long-nosed bat	*Leptonycteris sanborni*	*Cryptococcus diffluens*
Phyllostomidae	Lesser long-nosed bat	*Leptonycteris sanborni*	*Cryptococcus laurentii*
Phyllostomidae	Lesser long-nosed bat	*Leptonycteris sanborni*	*Cryptococcus parapsilosis*
Phyllostomidae	Lesser long-nosed bat	*Leptonycteris sanborni*	*Torulopsis glabrata*
Phyllostomidae	Orange nectar bat	*Lonchophylla robusta*	*Histoplasma capsulatum*
Phyllostomidae	Common sword-nosed bat	*Lonchorhina aurita aurita*	*Histoplasma capsulatum*
Phyllostomidae	Waterhouse's leaf-nosed bat	*Macrotus waterhoussi minor*	*Histoplasma capsulatum*
Phyllostomidae	Little big-eared bat	*Micronycteris megalotis*	*Histoplasma capsulatum*
Molossidae	None	*Molossus major*	*Histoplasma capsulatum*
Mormoopidae	Ghost-faced bat	*Mormoops megalophylla*	*Histoplasma capsulatum*
Molossidae	None	*Molossus major*	*Candida albicans*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Histoplasma capsulatum*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Malassezia furfur*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Malassezia globose*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Malassezia pachydermatis*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Malassezia sympodialis*
Molossidae	Pallas's mastiff bat	*Molossus molossus*	*Wangiella dermatitidis*
Molossidae	Black mastiff bat	*Molossus rufus*	*Histoplasma capsulatum*
Vespertilionidae	Silver-tipped myotis	*Myotis albescens*	*Wangiella dermatitidis*
Vespertilionidae	California myotis	*Myotis californicus*	*Histoplasma capsulatum*
Vespertilionidae	California myotis	*Myotis californicus*	*Pneumocystis* sp.
Vespertilionidae	Black myotis	*Myotis nigricans*	*Candida parapsilosis*
Vespertilionidae	Western long-eared myotis	*Myotis septentrionalis*	*Geomyces* sp.
Vespertilionidae	Indiana bat	*Myotis sodalist*	*Geomyces sp.*
Vespertilionidae	Fringed myotis	*Myotis thysanodes*	*Cryptococcus diffluens*
Natalidae	Mexican funnel-eared bat	*Natalus stramineus*	*Histoplasma capsulatum*
Natalidae	Mexican funnel-eared bat	*Natalus stramineus*	*Pneumocystis* sp.
Noctionidae	Southern bulldog bat	*Noctilio labialis*	*Histoplasma capsulatum*
Vespertilionidae	Common noctule	*Nyctalus noctule*	*Histoplasma capsulatum*
Emballonuroidea	Hairy slit-face bat	*Nycteris hispida*	*Histoplasma capsulatum*
Vespertilionidae	Eastern pipistrelle	*Perimyotis subflavus*	*Geomyces* sp.
Phyllostomidae	Pale speared-nosed bat	*Phyllostomus discolor*	*Histoplasma capsulatum*

(*Continued*)

TABLE 14.1 (Continued)

Bat family	Bat common name	Bat species	Fungus
Phyllostomidae	Pale speared-nosed bat	*Phyllostomus discolor*	*Wangiella dermatitidis*
Phyllostomidae	Greater spear-nosed bat	*Phyllostomus hastatus*	*Histoplasma capsulatum*
Mormoopidae	Lesser naked-backed bat	*Pteronotus davyi*	*Histoplasma capsulatum*
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	*Histoplasma capsulatum*
Mormoopidae	Parnell's mustached bat	*Pteronotus parnelli*	*Pneumocystis*
Mormoopidae	Parnell's mustached bat	*Pteronotus parnellii rubiginosa*	*Histoplasma capsulatum*
Mormoopidae	Wagner's mustached bat	*Pteronotus psilotis*	*Cryptococcus neoformans*
Mormoopidae	Big naked-backed bat	*Pteronotus suapurensis*	*Histoplasma capsulatum*
Phyllostomidae	Dwarf little fruit bat	*Rhinophylla pumilio*	*Candida* sp.
Phyllostomidae	Dwarf little fruit bat	*Rhinophylla pumilio*	*Kluyveromyces* sp.
Phyllostomidae	Dwarf little fruit bat	*Rhinophylla pumilio*	*Trichosporon beigelli*
Rhinolophidae	Lesser mouse-tailed bat	*Rhinopoma hardwickii*	*Basidiobolus ranarum Eidam*
Rhinolophidae	Lesser mouse-tailed bat	*Rhinopoma hardwickii*	*Blastomyces dermatitidis*
Rhinolophidae	Lesser mouse-tailed bat	*Rhinopoma hardwickii*	*Candida* sp.
Rhinolophidae	Lesser mouse-tailed bat	*Rhinopoma hardwickii*	*Trichosporon cutaneum*
Pteropodidae	Egyptian rousette	*Rousettus aegyptiacus*	*Encephalitozoon hellum*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Candida curvata*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Candida krusei*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Candida parapsilosis*
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Kluyveromyces* sp.
Phyllostomidae	Common yellow-shouldered bat	*Sturnira lilium*	*Wangiella dermatitidis*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Candida* sp.
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Cladosporium* sp.
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Coccidioides immitis*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Cryptococcus neoformans*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Histoplasma capsulatum*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Microsporum gypseum*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Pneumocystis*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Sporotrichum* sp.

Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Trichophyton mentagrophytes*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Trichophyton rubrum*
Molossidae	Brazilian free-tailed bat	*Tadarida brasiliensis*	*Trichophyton terrestre*
Molossidae	Broad-eared bat	*Tadarida laticaudata yucatanica*	*Histoplasma capsulatum*
Phyllostomidae	Great round-eared bat	*Tonatia bidens*	*Histoplasma capsulatum*
Phyllostomidae	White-throated round-eared bat	*Tonatia silvicola*	*Candida* sp.
Phyllostomidae	White-throated round-eared bat	*Tonatia silvicola*	*Kluyveromyces* sp.
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	*Candida guilliermondii*
Phyllostomidae	Tent-making bat	*Uroderma bilobatum*	*Torulopsis* sp.
Phyllostomidae	Heller's broad-nosed bat	*Vampyrops helleri*	*Candida parapsilosis*
Phyllostomidae	Heller's broad-nosed bat	*Vampyrops helleri*	*Trichosporon pullulans*

greater degree of genetic diversity (Taylor *et al.* 2012). Sexual reproduction, recombination, and genetic exchange occur in at least some of the *H. capsulatum* groups and may provide the means for diversification and adaptation to new hosts.

While *Histoplasma* was originally found between 45°N and 35°S latitudes, it is currently present throughout the Western Hemisphere, including Alberta, Canada, and other regions of the world. It is most common in microfoci in the Ohio, St. Lawrence, and Mississippi River valleys of the US (60–90% of the reported infections) and is the most common endemic mycosis in the US and the second most common systemic fungal disease of cats. It is also prevalent in several countries in South America in addition to been found in travelers to Central and South American countries or Caribbean islands, including the Dominican Republic, Guatemala, Colombia, Peru, and Nicaragua, as well as the Caribbean (Gascón *et al.* 2000). Infection typically occurs after activities that disturb the soil of old bird roosting sites or the moist entrances of bat-inhabited caves, although two Spanish teachers collaborating in a rural school appear to have been infected by moving old books in a closed room, and two other travelers, from sleeping on the floor in a wet forest region (Gascón *et al.* 2000; Jülg *et al.* 2008). The colonial habitats formed by large numbers of some species of bats maintain temperatures of 28–30 °C and humidity exceeding 60%, plus the nutrients present in guano, provide the necessary conditions for *H. capsulatum*'s life cycle (Taylor *et al.* 2012).

14.1.1.1 **Histoplasma capsulatum *in humans*** *H. capsulatum* is responsible for an acute pulmonary disease that may be severe and disseminated in immunocompromised people but is typically milder in those with intact immune responses. A case study of six infected, normal and healthy travelers did, however, detect multiple, diffuse, nodular and bilateral thoracic lesions, some with central cavitation, hilar adenopathy, and a non-calcified granuloma as well as diffuse inflammatory bronchial mucosa (Gascón *et al.* 2000). The yeast form is able to remain viable for years in the central area of calcified lesions and undergo recrudescence. Infected bats rarely develop severe disease.

Humans are believed to become infected by inhalation of aerosolized microconidia and hyphal fragments from fungal mycelia which develop and thrive in environmental conditions where soil is enriched by droppings in open areas under large bird roosts, particularly gregarious birds (starlings, blackbirds, chickens, oilbirds, and pigeons) and in bat guano from confined spaces like caves, mines, and buildings. Small distances between the guano deposits and ceilings are risk factors for cave bats that have a greater chance of inhaling a high concentration of spores (Taylor *et al.* 1999). The yeast form is, however, also able to infect experimental animals by the intranasal route (reviewed by Klite & Diercks 1965). It resides in the lungs as an intracellular parasite of pulmonary phagocytes. In addition to birds, bats, and humans, baboons, badgers, northern sea otters, raccoons, horses, dogs, and cats may be naturally infected with this fungus (reviewed in Teixeira *et al.* 2016).

Histoplasmosis was first linked to soil contaminated by house-dwelling *Eptesicus fuscus* in the northeastern US in 1958 (Emmons 1958). Other early reports associated this disease with guano-containing soil in Trinidad and Panama or with visitation to caves housing bats in Venezuela, Peru, Mexico, and South Africa (reviewed by Klite & Diercks 1965). People at high risk of infection included those involved in mining, exploration, and collection of guano (reviewed by Taylor *et al.* 2005).

A recent literature review revealed that, in the US between 1938 and 2013, *H. capsulatum* was responsible for 105 outbreaks of histoplasmosis and 2850 cases in 26 states and Puerto Rico (Benedict & Mody 2016). This may be an underestimate since histoplasmosis is not a reportable disease. The reports indicate that the early outbreaks were most frequently associated with exposure to buildings, chicken coops, farms without chicken coops, or other outdoor areas. These outbreaks, however, involved a lower number of cases than those occurring in other settings. Of these outbreaks, the majority of those associated with farms or chicken coops occurred in 1943–1969 and the last occurred in 1985. Citywide wind-borne outbreaks had the highest median number of cases. They were linked to environmental disturbances, including those occurring at a stream bank, a golf course, and either an abandoned amusement park or a tennis complex (Benedict & Mody 2016).

Bats or their droppings were associated with 23% of the outbreaks and birds or their droppings in 56% of the outbreaks. Disturbance of bird or bat droppings was found in 40% of the outbreaks; soil or plant matter disruption in 32% and 20%, respectively; and demolition or construction in 25% (Benedict & Mody 2016). Work-related exposures occurred in 33% of the outbreaks and birds, bats, or their droppings were reported in 86% of these cases. Two large outbreaks were school-related, however, most of the cases in remaining outbreaks occurred in adults. The states with the highest reported incidence of histoplasmosis were: Indiana (28%), Ohio (15%), Iowa (8%), Michigan (6%), Illinois (5%), Nebraska (5%), and Arkansas (5%). The majority of outbreaks (72%) began between May and November. For the outbreaks in which precise data were available, 14.7% of the patients were hospitalized ($n=1801$) and 1.1% died ($n=232$). In general, the rates of hospitalizations and deaths decreased over time (Benedict & Mody 2016).

14.1.1.2 **Histoplasma capsulatum *in bats in Mexico*** Two *H. capsulatum* groups have been isolated from hairy fruit-eating bats (*Artibeus hirsutus*) in Mexico: LAm A1 and Lam2 bat-associated clades. A bat-associated clade (BAC1) is also associated with the migratory bat species *Tadarida brasiliensis* and *Mormoops megalophylla* (Teixeira *et al.* 2016).

H. capsulatum was isolated from gut, lung, liver, and spleen samples of 8.2% of captured bats from 5 families, 13 genera, and 18 species in Mexico ($n=208$; 103 males and 105 females) (Taylor *et al.* 1999). All of the infected bats were adults, six males and eleven females. Affected species were as follows: 6.3% of *Pteronotus parnellii* ($n=16$), 40% of *Natalus stramineus* ($n=5$), 66.7% of *A. hirsutus* ($n=15$), 22.2% of *Leptonycteris nivalis* ($n=9$), 33.3% of *Myotis californicus* ($n=3$), and 14.3% of *M. megalophylla* ($n=7$) (Taylor *et al.* 1999). Yeast-phase fungi were seen within intra-alveolar and septal pulmonary macrophages. Fungal isolates were recovered from the lung ($n=8$), gut ($n=7$), liver (n=2), and spleen ($n=1$).

Using a 210-base pair fragment of the fungal *Hcp100* molecular marker, *H. capsulatum* DNA was detected from 81.6% of lung samples from young male *T. brasiliensis* migratory bats in a nonreproductive stage from Mexico (78.8% positive; $n=66$ bats) and Argentina (90.4% positive; $n=21$). Most of the Mexican and Argentinean bat lung samples were 99% similar in this region of DNA. Of note, *H. capsulatum* was only isolated from 3 of 87 lung samples, suggesting a low fungal burden in this tissue (González-González *et al.* 2012). This molecular means of detection is very specific and sensitive, even though fungal isolation is considered to be the gold standard for detecting fungal

infection. Isolation's major limitations are its low sensitivity and contamination of environmental samples with faster-growing microorganisms. While the rate of fungal isolation from bat droppings, contaminated soil, or infected bats is typically low, the infection rate of organs from bats in Mexico has been reported to be 66%. *T. brasiliensis* and other species of colonial bats having extensive ranges are believed to be particularly important in the spread of *H. capsulatum* over wide geographical areas, especially since *T. brasiliensis* is one of the most common bat species in the Western Hemisphere and may share its roosts with other migratory or nonmigratory bat species (Taylor *et al.* 2012).

Polymorphic DNA patterns in isolates in four of the fungus's key genes (*arf*, *H-anti, ole*, and *tub1*) allow its division into three groups in Mexico (Taylor *et al.* 2005). Group I includes primarily isolates from *T. brasiliensis* and a single clinical strain, group II is composed of two isolates from a single *T. brasiliensis* bat, and group III is composed of the human G-217B reference strain from the US. Group II differed so much from the other two groups that it may be considered a separate *H. capsulatum* population. One key 240-nucleotide microsatellite indicated an average DNA mutation rate of 2.39×10^{-9} substitutions per site per year (Taylor *et al.* 2012).

14.1.1.3 **Histoplasma capsulatum *in bats in Central America*** *H. capsulatum* was first isolated from a bat (*Pteronotus rubiginosa*) in Panama in 1962 (Shacklette *et al.* 1962). A separate study was conducted from 1965 to 1967 by the same research group in Panama to identify specific bat species infected by *H. capsulatum*. This study collected bats from a wide range of habitats, including caves, sea-caves, railroad culverts, and rainforests. The following bat species were found to be infected: 1.2% of *Carollia perspicillata* ($n=852$), 3.2% of *Desmodus rotundus* ($n=31$), 2.7% of *Glossophaga soricina* ($n=773$), 16.7% of *Lonchophylla robusta* ($n=24$), 25% of *Lonchorhina aurita* ($n=4$), 0.9% of *Molossus* species ($n=111$), 62.5% of *Noctilio labialis* ($n=8$), 33.3% of *Phyllostomus discolor* ($n=6$), 18.6% of *Phyllostomus hastatus* ($n=118$), 25.1% of *P. rubiginosa* ($n=1124$), 12.1% of *Pteronotus suapurensis* ($n=33$), 3.4% of *Tadarida yucatanica* ($n=89$), and 14.3% of *Tonatia bidens* ($n=7$) (Shacklette & Hasenclever 1969). Positive species included frugivorous, insectivorous, carnivorous, and hematophagous bats. Liver, spleen, lung, and intestinal contents harbored the fungus and a lower prevalence of infection was found in the kidneys. Interestingly, the rates of infection differed among different colonies of the same species. In the case of *P. rubiginosa*, this ranged from less than 7% at several sites to greater than 35% at others. Prevalence of infection was lower in house bats, perhaps due to high temperatures on sunny days in the attics where the bats roosted. The increase in bat body temperature may decrease the rate of infection since the yeast form of this fungus is temperature-sensitive (Shacklette & Hasenclever 1969).

A study conducted in 1963 in the Republic of Panama and the Canal Zone cultured *H. capsulatum* from 10.0% of organs from six genera of bats with differing dietary habits ($n=623$) (Klite & Diercks 1965). The species of positive bats are as follows: 5% of *Carollia perspiculata* ($n=141$), 25% of *Chilonycteris rubiginosa* ($n=120$), 3.5% of *G. soricina* ($n=57$), 22.6% of *Micronycteris megalotis* ($n=84$), 1% of *Molossus major* ($n=99$), and 15.8% of *P. hastatus* ($n=19$). The sites of fungal isolation varied among bat species: *C. perspiculata* (lungs, liver and spleen, but not feces or kidney), *C. rubiginosa* (frequent presence of large amounts of fungi were found in the feces and less frequently found in the lungs, liver, spleen, and kidneys), *G. soricina* (spleen), *M. megalotis* (primarily lungs, but

also feces, spleen, and kidneys), *M. major* (lungs and spleen), and *P. hastatus* (lung and spleen) (Klite & Diercks 1965). An earlier study of the region by the same group of researchers cultured *H. capsulatum* from 2% of tested bat livers and spleens ($n=549$). In addition to *Carollia*, *Chilonycteris*, and *Glossophaga*, fungus was cultured from the *Desmodus* and *Lonchorhina* genera. No gross histopathologic lesions were seen in the bat organs, but inflammatory foci with numerous eosinophils were seen in the intestinal tract in almost all of the infected bats, as well as in the peribronchial stroma of the lungs, the hepatic sinusoids, and the interstitial tissue of the kidneys in some animals, but the yeast form of *H. capsulatum* was not detected (Diercks *et al.* 1965).

In the late 1990s, two groups of people visited a cave in Costa Rica inhabited by bats. Afterwards, acute pulmonary histoplasmosis developed in 72% of those from one group ($n=75$) and 64% of the other group ($n=14$). The most common symptoms were headache, fever, cough, myalgia, chest pain, nausea, and dyspnea. *H. capsulatum* was present in the cave's bat guano. Accordingly, crawling increased the risk of infection while handwashing before eating decreased risk, but wearing loose-fitting paper masks did not (Lyon *et al.* 2004).

In El Salvador, *H. capsulatum* was cultured from 19.2% of lung, liver, spleen, or feces of *Artibeus jamaicensis* ($n=52$) and 12.5% of *P. discolor* ($n=8$) (Klite 1965). Infections have also been reported in *T. brasiliensis* from Trinidad and *Macrotus waterhoussi minor* from Cuba (reviewed by Taylor *et al.* 1999). No fungi were found in 178 bats of six different species in Bolivia, however.

14.1.1.4 **Histoplasma capsulatum *in bats in South America*** A study of 1001 Columbian bats representing 31 species with different dietary and roosting habits and several different ecologic zones was able to culture *H. capsulatum* from 0.3% of the bats: from the lungs, liver, and spleen of one *C. perspicillata* from a rainforest and from the spleens of one *D. rotundus* from a cultivated valley and one *Eptescicus brasiliensis* from a forested mountain region (Tesh *et al.* 1968). *H. capsulatum* was also isolated from the spleen and liver of a *Eumops bonariensis* in Argentina (Canteros *et al.* 2005).

In the major population region around São Paulo, Brazil, 3.6% of the spleens or livers of tested bats ($n=2427$) were culture-positive for *H. capsulatum* (Dias *et al.* 2011). All of bat species were from the insectivorous Molossidae family ($n=1391$), primarily *Molossus molossus* and *Nyctinomops macrotis*, but also one *T. brasiliensis, Molossus rufus*, and *Eumops glaucinus*. Significantly more females than males were infected. Most of the bats caught on the ground were ill (28.7% of 87 positive bats), while the other bats appeared to be healthy. No infection was detected in Phyllostomidae (812 bats tested), Vespertilionidae (203 bats), or Emballonuridae (one bat).

H. capsulatum and *Pneumocystis* species both infect human lung tissue, which may result in severe disease in immunocompromised people. DNA from lung tissue of bats from Argentina, French Guiana, and Mexico were tested for the prevalence of co-infection with these two fungi. Infection with only *H. capsulatum* was seen in 45.1% of the bats ($n=122$), 6.6% were infected with only *Pneumocystis* species, and 35.2% were co-infected (González-González *et al.* 2014). The bat species positive for either fungus were as follows: *A. hirsutus* (100% positive for *Histoplasma*, 60% for *Pneumocystis*: $n=5$), *C. perspicillata* (100% positive for *Histoplasma*: $n=1$), *G. soricina* (43.8% positive for *Histoplasma*, 56.3% for *Pneumocystis*; $n=16$), *N. stramineus* (62.5% positive for *Histoplasma*, 12.5% for *Pneumocystis*; $n=8$), *Pteronotus davyi* (100% positive for

Histoplasma; $n=1$), *P. parnellii* (66.7% positive for *Histoplasma*, 33.3% for *Pneumocystis*; $n=8$), *M. megalophylla* (100% positive for *Histoplasma*; $n=3$), *T. brasiliensis* (86.9% positive for *Histoplasma*, 42.9% for *Pneumocystis*; $n=84$), and *M. californicus* (100% positive for *Histoplasma*, 100% for *Pneumocystis*; $n=1$) (González-González *et al.* 2014). Prevalence of infection with *H. capsulatum* may be linked to bat colony size and their movements, while prevalence for *Pneumocystis*, to crowding and migration (reviewed by González-González *et al.* 2014). It is not known whether co-infection with these two fungi leads to differences in pathology or intra- or interspecies fungal transmission.

14.1.1.5 Histoplasma capsulatum *in bats in Africa*

H. capsulatum var. *duboisii* is the causative agent of African histoplasmosis. It is found in the western and central areas of Sub-Saharan Africa and Madagascar, especially in HIV-positive people. This fungus was isolated from 17.8% of samples of soil mixed with bat guano ($n=45$) as well as the intestinal contents of one *Nycteris hispida* bat in Nigeria ($n=35$) (Gugnani *et al.* 1994).

14.1.1.6 Histoplasma capsulatum *in bats in Eurasia*

While typically found in tropical and sub-tropical regions, autochthonous human *H. capsulatum* histoplasmosis (the Eurasian clade) has been reported in Italy, Germany, Turkey, Egypt, England, the Netherlands, and Poland as well as in skin, lymph nodes, and internal organs of badgers from Denmark, Austria, and Germany (reviewed by González-González *et al.* 2013 and Teixeira *et al.* 2016). It is also present in Thailand, China, and India. A study in France used a specific molecular marker, a fragment of a highly-specific co-activator protein-coding gene (*Hcp100*), to determine whether *H. capsulatum* was also present in European bat populations. Lung samples from 83 wild or captive bats representing five species were tested. All tested bats had been found dead. While this *H. capsulatum*-specific DNA marker was only found in one sample of a *N. noctula* bat ($n=18$) (González-González *et al.* 2013), it demonstrates that *H. capsulatum* is also found in European bats.

14.1.2 *Blastomyces dermatitidis*

Blastomyces dermatitidis is the causative agent of blastomycosis, characterized by granulomatous inflammation of the lungs, but may involve the skin, bones, eyes, central nervous system, mammary tissue, and the male reproductive tract. It is a dimorphic fungus with free-living mycelial stages in soil or decaying organic matter that produces spores that are inhaled by a host before transitioning into the yeast form within pulmonary alveoli. This fungus is endemic in the Ohio, Mississippi, and Missouri river basins in the US in the acidic soils close to and within river basins (reviewed by Raymond *et al.* 1997).

B. dermatitidis was cultured from the lungs of one of 155 and the liver of one of 46 lesser mouse-tailed bats (*Rhinopoma hardwickei hardwickei*) from an abandoned schoolhouse and the basement of an historical tomb in India, respectively (Khan *et al.* 1982; Randhawa *et al.* 1985). The infected bats appeared to be healthy and no lesions were found in their visceral organs, including the liver, although this fungus was pathogenic to white mice. No *B. dermatitidis* was found in samples of bat guano ($n=46$). When *R. hardwickii* was infected orally with *B. dermatitidis*, low numbers of fungi survived the trip through the gastrointestinal tract and into the feces. It was, however, cultured from the stomach, intestine, and feces after 16–-24 h and remained in the rectum up to 48 h post-infection (Chaturvedi *et al.* 1986).

Pulmonary blastomycosis has also been observed in a dead, pregnant *P. giganteus* (Raymond *et al.* 1997). Both lungs contained multiple firm, white-gray, coalescing nodules of various sizes. T lymphocytes are an important component in protection against pathology and immunocompromised hosts are more susceptible to infection. Macrophages, activated by cytokines from T lymphocytes, restrict growth of the yeast. Since pregnancy leads to suppression of maternal T cell-dependent immunity, this fruit bat may have been more susceptible to severe infection. The bat's lungs contained large numbers of necrotic neutrophils which may have also stimulated the growth of *B. dermatitidis* (Raymond *et al.* 1997).

14.1.3 *Pneumocystis*

Pneumocystis species are highly diversified and infect a wide range of mammals. They are transmitted between hosts by the airborne route. They are extracellular fungi that often attach to type I pneumocytes. These fungi have a high level of host-species-related diversity typically present in microbes with close host specificity. The high degree of fungal divergence is likely to be due to the prolonged process of co-evolution associated with co-speciation with their respective hosts (reviewed by González-González *et al.* 2014). Several *Pneumocystis* species infect humans and may result in severe to fatal pneumonia, especially in the immunocompromised segments of the population, such as those who are HIV-positive.

14.1.4 *Coccidioides*

Coccidioides posadasii is a causative agent of coccidioidomycosis, a potentially serious infection in humans and animals. It is endemic to Northeast Brazil, where it has caused disease in humans and is present in soil. A study of Brazilian bat lungs, spleens, and livers from *C. perspicillata* bats produced mold colonies on agar (Cordeiro *et al.* 2012). These organs also were observed to contain fungal spherules with endospores upon histopathologic analysis. Additionally, coccidioidal antibodies and antigens were present in the lungs or liver of *G. soricina* and *D. rotundus* bats.

14.1.5 *Encephalitozoon*

Encephalitozoon hellem is an obligate intracellular parasite that may cause severe disease in immunocompromised people, including respiratory, urogenital, and disseminated diseases. Other members of this genus are also pathogenic to humans. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* may lead to diarrhea and chronic wasting and *Encephalitozoon cuniculi* commonly infects the central nervous and respiratory systems, in addition to the digestive system. *E. hellem* is also carried by birds, some of which develop clinical disease. Transmission to humans is believed to result from consumption of food or water contaminated with bird droppings. Disseminated microsporidiosis due to infection with *E. hellem* also was seen in a dead adult female Egyptian fruit bat (*R. aegyptiacus*) in an enclosed, crowded indoor facility in an American zoo (Childs-Sanford *et al.* 2006). Gross observation revealed poor body condition, bilateral enlargement of the kidneys, and mottling of the liver. Inflammatory lesions were particularly pronounced in the urogenital tract and liver and were associated with intracytoplasmic microsporidian spores (Childs-Sanford *et al.* 2006).

T lymphocytes are believed to be a crucial component in protection against microsporidial infection. Macrophages appear to disseminate infection rather than eliminate these parasites (reviewed by Childs-Sanford *et al.* 2006).

14.1.6 Other fungi of bats

Basidiobolus ranarum Eidam was found in intestinal contents of 7% ($n = 200$) of lesser rat-tailed bats (*R. h. hardwickei Gray*) from the Delhi area of India. No macroscopic or microscopic lesions were found in the intestines of the bats (Chaturvedi *et al.* 1984).

The dermatiaceous fungi *Wangiella dermatitidis* was isolated from the internal organs of *P. discolor, M. molossus, Sturnira lilium*, and *Myotis albescens* in Brazil (Reiss & Mok 1979).

14.2 BROAD SURVEYS OF FUNGI IN BATS

14.2.1 Asia

A survey of fungi was conducted in a karst cave, the Heshang Cave in China (Man *et al.* 2015). This is a pristine carbonate cave with a pH of 8.2–8.7 with extremely low concentrations of mineral nutrients. Fungi were found growing in or on bat guano, sediments, and weathered rocks. The guano was sampled from a dark area of the cave. All of the fungi from the guano were members of the phylum Ascomycota. The predominant order of fungi was Eurotiales (89% of the isolates), followed by Hypocreales (8%) and Glomerellales (2%). Six genera of fungi were cultured: 52% were from the *Penicillium* genus, 37% were *Aspergillus*, 2% were *Acrostalagmus*, 2% were *Beauveria*, 2% were *Oidiodendron*, 2% were *Fusarium*, and the remaining 5% were unclassified (Man *et al.* 2015). Fungal diversity was the lowest in guano and highest in sediments.

14.2.2 Europe

Airborne fungi were collected at the beginning, middle, and end of a hibernation period by bats in a large man-made underground bat reserve in Poland (Kokurewicz *et al.* 2016). In November, bat numbers were highest (1167 individuals of 7 taxa) as was the number of fungal species (34) and spore count (628.5 colony-forming units, CFU/1 m^3 of air). Numbers of bats dropped slightly in January (956 individuals of 7 taxa) as did spore numbers (579.4 CFU/1 m^3 of air) but not numbers of fungal species. The lowest numbers of bats were seen in March (366 individuals of 5 taxa) and also the lowest number of spores (199.4 CFU/1 m^3 air). The density of airborne fungi isolated from the underground area ranged between 20 CFU and 1198 CFU while that of the control outdoor air samples was from 102 CFU to 242 CFU, contrary to other reports that found fungal spore number higher in the external environment. There was a positive relationship between number of bats and fungal concentration, however, correlation does not necessarily indicate causation. The World Health Organization suggests that spore concentrations up to 1500 CFU in 1 m^3 air are acceptable for human health if it is a mixture of species. The spore levels in this study were far beneath that number. During the entire

survey period, the underground reserve housed 9 bat taxa, 32 airborne filamentous fungi, and 2 air-borne yeasts: *Absidia glauca*; members of the *Alternaria alternata* complex; *Alternaria botrytis; Aspergillus* species sections *Nigri, Flavi*, and *Fumigati; Aspergillus* species 1 section *Circumdati; Aspergillus* species 2 section *Circumdati; Candida albicans*; members of the *Chaetomium globosum* complex, *Cladosporium cladosporioides* complex, and *Cladosporium herbarum* complex; *Clonostachys rosea*; members of the *Fusarium oxysporum* complex; *Mucor flavus, Mucor hiemalis; Mucor luteus; Mucor racemosus; Paecilomyces fumosoroseus*; members of the *Paecilomyces variotii* complex; *Penicillium* species 1, 2, and 3 section *Chrysogena; Penicillium* species 1 and 2 section *Citrina; Penicillium* species section *Exilicaulis; Phoma* species; *Pseudogymnoascus destructans; Rhizopus stolonifer; Rhodotorula rubra; Sarocladium strictum; Trichoderma harzianum*; and non-sporulating white and black colonies (Kokurewicz *et al.* 2016). Members of the *C. cladosporioides* complex were the most frequently isolated fungi from samples taken both outside and underground in November, but in January, were only present in samples taken inside. *Penicillium* species 1, section *Chrysogena* was most frequently isolated outside and underground in March and from outside samples in January (Kokurewicz *et al.* 2016). Analysis of airborne fungal presence and number provides a noninvasive means of testing that is less likely to disturb bats, especially during their critical hibernation period. This method opens up studies that are not otherwise possible.

14.2.3 The Americas

14.2.3.1 United States In order to determine whether bat guano could harbor pathogenic fungi, 371 fresh guano samples were collected from ten sites in four caves in southwestern US (Kajihiro 1965). The majority of the samples (53.9%; $n = 371$) were obtained from Carlsbad Caverns, which housed approximately 300 000 Mexican free-tail bats (*Tadarida mexicana*) during the collection period. Surprisingly, several species of dermatophytes were found in the guano. *Microsporum gypseum* was isolated at all sites (incidence of infection = 22.4%), *Trichophyton mentagrophytes* from 70% of the sites (incidence = 5%), *Trichophyton rubrum* from 30% of the sites (incidence = 3%), and *Trichophyton terrestre* at 10% of the sites (incidence = 0.5%). When pooled liver-spleen-lung samples were tested, 10% contained *H. capsulatum* and 1.7% contained *Trichophyton mentagrophytes*. The following human pathogenic fungi were also isolated: *Candida* species, *Cladosporium* species, *Coccidioides immitis, Cryptococcus neoformans*, and *Sporotrichum* species (Kajihiro 1965).

14.2.3.2 South America In a large study of fungi and bats from the Amazon Basin of Brazil, bat livers, spleens, and lungs of 4.4% of bats were found to carry fungi. The fungi in the organ samples included *Candida* species (*Candida parapsilosis, Candida guilliermondii, C. albicans, Candida scottii, Candida solani, Candida stellatoidea, Candida pseudotropicalis, Candida brumptii, Candida claussenji*, and *Candida curvata*) in 4.3% bats, *Trichosporon* (*Trichosporon pullulans* and *Trichosporon beigelii*) in 1.1%, *Torulopsis* (*Torulopsis famata, Torulopsis candida, Torulopsis dattila, Torulopsis colliculosa, Torulopsis glabrata, Torulopsis inconspicua*, and *Torulopsis aeria*) in 0.9%, *Kluyveromyces* species in 0.4%, and *Geotrichum* species in less than 0.1% (Mok *et al.* 1982).

These fungi include several pathogenic species: *C. parapsilosis, C. guilliermondii, C. albicans, C. stellatoidea, C. pseudotropicalis, T. beigelii*, and *T. glabrata* (Mok *et al.* 1982). The bat species from which fungi was recovered were as follows: *Saccopteryx bilineata, V. helleri, R. pumilio, G. soricina, Carollia castanea, U. bilobatum, Sturnira tildae, A. lituratus, T. silvicola, S. lilium, Mesophylla macconelli, Myotis nigricans, Lionycteris spurrelli, Peropteryx macrotis, A. jamaicensis, Trachops cirrhosis, A. cinereus, C. perspicillata, M. molossus, Chiroderma villosum, D. rotundus, P. hastatus, P. parnellii, Phyllostomus* species, *Nyctinomops laticaudatus, Centronycteris maximiliani*, and *Micronycteris* species (Mok *et al.* 1982). The association of fungi with bats showed no geographical or seasonal pattern, but appeared to be related to vegetation in the bat habitat. Two-thirds of positive bats were from deforested areas overgrown with secondary vegetation or transformed into open fields, orchards, cattle ranches, or cultivated plots and the remaining bats were from dense virgin forests (Mok *et al.* 1982).

Other fungi recovered from bat organs in North or South America included: *C. neoformans* from *C. perspicillata* and *Pteronotus psilotis* bats; *C. parapsilosis, C. diffluens, C. albidus, C. laurentii*, and *T. glabrata* from *Leptonycteris sanborni* bats; and *C. diffluens* from *Myotis thysanodes* bats (Grose & Marinkelle 1966; DiSalvo *et al.* 1969). *Sporothrix schenckii* was additionally isolated from bat feces and *Microsporum canis* from their fur.

14.2.4 Fungi inhabiting bat external surfaces

Given the enormity of the threat of white-nose syndrome (WNS) to bats, it is important to learn what other fungi are present on bat external surfaces prior to their exposure to *Pseudogymnoascus destructans*. Fifty-three operational taxonomic units were detected from bats' wings (Johnson *et al.* 2013). A wide range of diverse fungi, including *Geomyces* isolates, are present on bats prior to the arrival of WNS. Members of the Ascomycota phylum comprised the majority of the fungal isolates. The most common orders in this phylum were members of Helotiales (14%), Eurotiales (13%), Capnodiales (12%), and Hypocreales (11%) and the most common Ascomycota genera were *Cladosporium, Geomyces, Mortierella, Penicillium*, and *Trichosporon*. *Geomyces* isolates were placed in seven distinct clades, all of which are psychrotolerant, unlike the psychrophilic *P. destructans*. Most of these psychrotolerant *Geomyces* species were isolated from mildly damaged wings of *M. septentrionalis, M. sodalis,* and *P. subflavus* and were morphologically different from *P. destructans*. Basidiomycota composed 14% of the isolates, including members of Polyporales and Cystofilobasidiales (primarily *Trichosporon* species). Zygomycota composed 13% of the isolates, including the orders Mortierellales and Mucorales and the genera *Mortierella* and *Mucor* (Johnson *et al.* 2013).

In 2012, in the northern US, bats were found with grossly visible fungal skin infections. They appeared similar to those due to WNS, but were outside of its range at that time. A novel psychrophilic species of *Trichophyton, Trichophyton redellii,* was isolated in culture, by direct DNA amplification and sequencing, and visual impression (Lorch *et al.* 2015). DNA from the same fungus was subsequently found in bats from the southern part of the country. These findings indicate the presence of at least one more fungus responsible for skin lesions in North American bats.

14.3 EXPERIMENTAL INFECTION OF BATS WITH FUNGI

Natural fungal infection of *Artibeus lituratus* has not been reported, perhaps because they often roost in open areas, such as heavy foliage of large trees in dry forest areas. When *A. lituratus* were experimentally infected with viable yeast and mycelial particles of *Paracoccidioides brasiliensis* by the oral route, the fungi survived less than 8 h in the bats' digestive tracts (Greer & Bolaños 1997). Mycelial particles, the infective form of the fungi, were more susceptible to killing than the yeast and were killed before entering the rectum. Following intranasal infection of *A. lituratus* with a mixture of 10^6 *H. capsulatum* mycelia and spores *in vitro*, however, viable fungi were present in the lungs, liver, spleen, and gut by 2 weeks (McMurray & Greer 1979). The spread of fungi from the respiratory tract to the gut is important in its transmission to humans. None of the bats infected via the intranasal route died of pulmonary histoplasmosis despite extensive involvement of the lungs, although gross lesions were rarely found in the viscera. By contrast, intraperitoneal infection with only 10 viable *H. capsulatum* led to systemic disease in approximately half of the bats, including gross pathologic abnormalities and histologic lesions resembling chronic inflammation. Numerous viable fungi were present in the tissues. The spleen and liver were most often affected (McMurray *et al.* 1978; Greer & McMurray 1981b). The above findings suggest that natural exposure to *H. capsulatum* may result in chronic and disseminated, rather than acute, infection in this species of bat, and could possibly enable them to function as long-term reservoir hosts. It should be noted, however, the above reports represent experimental infection of a species of bat that is not known to be naturally infected. While no gross visceral lesions have been found in naturally infected bats, yeast are able to replicate in various tissues.

14.4 IMMUNE RESPONSE TO FUNGI

The bat immune system may play a major role in preventing pathology by *H. capsulatum* infection in at least some bat species following experimental infection via the oral or intranasal route. Circulating specific complement-fixing and precipitating antibodies were present at 3 and 5 weeks post-infection, respectively. Transient delayed type hypersensitivity (cell-mediated immunity) to the histoplasmin antigen appeared within 2 weeks, waned, and had almost disappeared by 6 weeks, while antibodies persisted for at least 9 weeks (McMurray & Greer 1979). It is noteworthy that cell-mediated immunity is believed be the major means of protection in previously sensitized humans. A separate study by the same group of researchers found that when *A. lituratus* were infected with *H. capsulatum*, the bats which received a high dose of fungi developed elevated levels of IgM and IgA beginning at 2 weeks and lasting until the study's end. IgG levels were not elevated until much later (8–9 weeks) (McMurray *et al.* 1982).

When *A. lituratus* were inoculated via the intraperitoneal route with 10^6 yeast phase *P. brasiliensis* fungi, they developed a fatal, disseminated disease that is similar to that found in humans (Greer & McMurray 1981a). Delayed type hypersensitivity appeared within 2 weeks, but no precipitating antibodies were detected for up to 7 weeks. After intranasal infection with 10^5 *P. brasiliensis* fungi, the resulting pulmonary disease spread to the lungs and spleen by 3 weeks and to the liver by 9 weeks. Even 10 viable fungi caused pulmonary disease. Antibodies appeared at 5 weeks and persisted for an additional

several weeks. No viable *P. brasiliensis* was recovered from the intestines or fecal contents of any of these bats. The lack of intestinal involvement suggests that these bats fail to serve a direct role in dissemination of this fungus (Greer & McMurray 1981a).

14.5 YEAST IN BATS

14.5.1 *Candida*

In a study of fungal species of Nigerian bats, yeasts of medical importance were recovered from the visceral organs of 15% of the animals ($n=120$) (Oyeka 1994). *C. albicans* was the most common fungus of the liver, spleen, kidneys, and intestinal contents of *Glossophaga soricinia* bats (25%; $n=40$) and all of the tested organs were covered by white patches of yeast colonies. *Candida krusei* was recovered from livers and spleens of 6% of *M. maior* ($n=50$), while 16.7% of lesser free-tailed bats (*R. h. hardwickei*) harbored *Trichosporon cutaneum* and *Candida* species ($n=30$) (Oyeka 1994).

Five *Candida* species yeasts were isolated from the feces of 12.3% of tested Brazilian urban bats ($n=57$): *C. guilliermondii, C. krusei, Candida lusitaniae, C. parapsilosis*, and *Candida pelliculosa* (Botelho *et al.* 2012). All of the positive samples could produce biofilms and additionally were only isolated from bats of the family Phyllostomidae: 16.7% of *S. lillium* ($n=18$), 5.9% of *Artibeus fimbriatus* ($n=17$), and 20% of *A. lituratus* ($n=15$). Fifteen days after intravenous inoculation of the yeast into Swiss mice, *Candida* could be isolated from the mice's kidneys. Infecting mice with 10^8 CFU of *C. guilliermondii* and *C. lusitaniae* led to a mortality rate of 75 and 50%, respectively, by 10 days post-infection (Botelho *et al.* 2012). In immunocompetent people, *Candida* often colonizes the gastrointestinal and reproductive tracts without causing pathology. However, they are important causative agents of fungal-related death in those who are immunocompromised and may infect the blood, urine, and oropharyngeal tract. The presence of *Candida* species in bats from an urban area may be problematic since bat guano can contaminate air conditioning systems in health care settings and infect immunocompromised patients, with severe consequences.

14.5.2 *Malassezia*

Malassezia genus yeasts may cause opportunistic infections of the skin and auricular canal of the ears of humans and animals, particularly dogs and cats. Members of this genera were isolated from the acoustic meatus of the ears of 80% of *M. molossus* in Brazil ($n=30$). The following *Malassezia* were present: *Malassezia pachydermatis* in 62.5% of the bats, *Malassezia furfur* in 20.8%, *Malassezia globosa* in 12.5%, and *Malassezia sympodialis* in 4.2% of the bats (Gandra *et al.* 2008).

14.5.3 Yeasts in Japan

Endogenous histoplasmosis is very rare in Japan. In a study of bat guano from 20 frequently explored caves in Japan, *Trichosporon* species were most commonly encountered: *Trichosporon laibachii* in seven caves and *Trichosporon porosum* from five caves (Sugita *et al.* 2005). Seven new *Trichosporon* species were also identified in 10 caves using molecular phylogenetic analysis. Additionally, eight species of ascomycetous

yeast were found (*Candida palmioleophila, C. lusitaniae, Debaryomyces hansenii, Hanseniaspora* spp., *Saccharomyces cerevisiae, Saccharomyces kluyveri, Williopsis californica*, and *Zygosaccharomyces florentinus*) as well as the basidiomycetous yeast *Cryptococcus podzolicus*. Some of these species may be pathogenic to immunocompromised people who visit caves (Sugita *et al.* 2005).

14.6 CONCLUSIONS

Histoplasma species, some of the most common agents of endemic mycosis, are primarily found in the Americas, but have also been reported in Eurasia and Africa. Their life cycles consist of an infective saprophytic mycelial phase in soil and an intracellular, parasitic yeast phase. Areas under large bird roosts or confined areas containing bat guano provide ideal growth conditions for the fungi. Humans entering these habitats risk infection by inhalation of microconidia or hyphal fragments. While infection in bats is rarely severe, the yeast may cause life-threatening pulmonary or disseminated disease in humans, particularly in immunocompromised people, but occasionally in immunocompetent persons as well. A Brazilian study also reported isolating the fungus from grounded bats.

Exposure to bats or their droppings was associated with 23% of the outbreaks in humans. *H. capsulatum* has been isolated from various organs, including the gut, lungs, and kidneys, of at least 32 species of bats of all dietary habits. Fungi are abundant in the fecal material from some, but not all, bat species. Prevalence of infection varies greatly among bat species and within species from different colonies as well and may be as high as 90% in some roosts. *T. brasiliensis*, as well as other colonial bats with large ranges, may be particularly important in spreading *H. capsulatum* over wide geographical areas, especially if sharing roosts with other bat species.

A substantial number of members of various bat species were co-infected with *H. capsulatum* and *Pneumocystis*. Several *Pneumocystis* species infect humans and cause severe to fatal pneumonia, particularly in HIV-positive people. The study did not report on the effects of this dual infection in affected bats or the risk to humans exposed to their droppings.

Infection with *Blastomyces dermatitidis* leads to granulomatous lung inflammation, but may affect other organ systems as well, including the central nervous system. Like *H. capsulatum, Blastomyces* are dimorphic fungi. Their free-living mycelial stage produces infectious spores that transform into parasitic yeast in the host's pulmonary alveoli. A dead, pregnant bat was also found with severe pulmonary blastomycosis, perhaps due to her immunocompromised condition.

Infection by either *Coccidioides posadasii* or *Encephalitozoon* species may result in severe disease in humans. These fungi have been detected in internal organs from several bat species. Disseminated disease due to infection by the latter group of fungi was also reported in a dead, captive *R. aegyptiacus*. Other fungi found in bats include *Basidiobolus ranarum Eidam* in India and *Wangiella dermatitidis* in Brazil. Yeasts that infect bat internal organs include several *Malassezia* species and *C. albicans*, which formed white colonies on the surface of bats' livers, spleens, and kidneys. In Japan, several ascomycetous and one basidiomycetous yeast species were found in guano from frequently explored caves.

In an underground bat reserve in Poland that harbored nine bat taxa, 32 species of filamentous fungi, and two species of yeasts, bat number corresponded to fungal spore concentration. While spore density was higher inside the reserve than in the external environment, it remained below that deemed to be unhealthy for humans. Similarly, a 1965 study of guano from caves in the southwestern US, primarily Carlsbad Caverns, home to large numbers of *T. mexicana*, detected the following species of fungi that are pathogenic to humans: *H. capsulatum, Candida* species, *Cladosporium* species, *Coccidioides immitis, Cryptococcus neoformans*, and *Sporotrichum* species. Despite the presence of these fungi in bat guano, Carlsbad Caverns is visited by large numbers of tourists each year and is not implicated as a risk area for contracting disease in immunocompetent people. A separate study of fungal diversity in internal organs of bats of Amazonia detected many different fungal species in about 4% of the bats. Several of these are also known human pathogens, such as various *Candida* species, *Trichosporon beigelii*, and *Torulopsis glabrata*.

Prior to the arrival of WNS in the US, a survey of bat external surfaces detected a wide variety of fungi, primarily from the Ascomycota phylum, including seven distinct clades of *Geomyces* which, unlike the psychrophilic *P. destructans*, were psychrotolerant. Another psychrophilic fungal species, however, was detected on bats in the northern, then the southern, US. This fungus, *Trichophyton redellii*, causes a grossly visible infection of bats that is similar to WNS. Other such surveys of bats' exteriors are sorely needed in order to determine whether this, or other, fungal pathogens are also becoming major threats to bat populations.

REFERENCES

Benedict K, Mody RK. 2016. Epidemiology of histoplasmosis outbreaks, United States, 1938–2013. *Emerging Infectious Diseases.* 22(3):370–378.

Botelho NS, de Paula SB, Panagio LA, Pinge-Filho P, Yamauchi LM, Yamada-Ogatta SF. 2012. *Candida* species isolated from urban bats of Londrina-Paraná, Brazil and their potential virulence. *Zoonoses and Public Health.* 59:16–22.

Canteros CE, Iachini RH, Rivas MC, Vaccaro O, Madariaga J, Galarza R, Snaiderman L, Martínez M, Paladino M, Cicuttin G, Varela E, Alcoba E, Zuiani F, Sahaza JH, Taylor ML, Davel G. 2005. First isolation of *Histoplasma capsulatum* from the urban bat *Eumops bonariensis*. *Revista Argentina de Microbiologia.* 37(1):46–56.

Carter DA, Taylor JW, Dechairo B, Burt A, Koenig GL, White TJ. 2001. Amplified single-nucleotide polymorphisms and a (GA)n microsatellite marker reveal genetic differentiation between populations of *Histoplasma capsulatum* from the Americas. *Fungal Genetics and Biology.* 34:37e48.

Chaturvedi VP, Randhawa HS, Khan ZU, Singh N, Kini S. 1984. Prevalence of *Basidiobolus ranarum Eidam* in the intestinal tract of an insectivorous bat, *Rhinopoma hardwickei hardwickei* Gray, in Delhi. *Sabouraudia.* 22(3):185–189.

Chaturvedi VP, Randhawa HS, Kini S, Khan ZU. 1986. Survival of *Blastomyces dermatitidis* in the gastrointestinal tract of an orally infected insectivorous bat, *Rhinopoma hardwickei hardwickei* Gray. *Journal of Medical and Veterinarian Mycology.* 24(4):349–352.

Childs-Sanford SE, Garner MM, Raymond JT, Didier ES, Kollias GV. 2006. Disseminated microsporidiosis due to *Encephalitozoon hellem* in an Egyptian fruit bat (*Rousettus aegyptiacus*). *Journal of Comparative Pathology.* 134:370–373.

Cordeiro RDA, de Castro e Silva KR, Brilhante RSN, Moura FBP, Duarte NFH, Marques FJDF, Cordeiro RDA, Filho REM, de Araújo RWB, Bandeira TDJPG, Rocha MFG, Sidrim JJC. 2012. *Coccidioides posadasii* infection in bats, Brazil. *Emerging Infectious Diseases.* 18(4):668–670.

Dias MAG, Oliveira RMZ, Giudice MC, Netto HM, Jordaõ LR, Grigorio IM, Rosa AR, Amorim J, Nosanchuk JD, Travassos LR, Taborda CP. 2011. Isolation of *Histoplasma capsulatum* from bats in the urban area of São Paulo State, Brazil. *Epidemiology and Infection.* 139:1642–1644.

Diercks FH, Shacklette MH, Kelley HB Jr, Klite PD, Thompson SW II, Keenan CM. 1965. Naturally occurring histoplasmosis among 935 bats collected in Panama and the Canal Zone, July 1961–February 1963. *American Journal of Tropical Medicine and Hygiene.* 14(6):1069–1072.

DiSalvo AF, Ajello L, Palmer JW Jr, Winkler WG. 1969. Isolation of *Histoplasma capsulatum* from Arizona bats. *American Journal of Epidemiology.* 89:606–614.

Emmons CW. 1958. Association of bats with histoplasmosis. *Public Health Reports.* 73:590–595.

Gandra RF, Gambale W, de Cássia Garcia Simão R, da Silva Ruiz L, Durigon EL, de Camargo LM, Giudice MC, Sanfilippo LF, de Araújo J, Paula CR. 2008. *Malassezia* spp. in acoustic meatus of bats (*Molossus molossus*) of the Amazon Region, Brazil. *Mycopathologia*. 165(1):21–26.

Gascón J, Torres JM, Luburich P, Ayuso JR, Xaubet A, Corachán M. 2000. Imported histoplasmosis in Spain. *Journal of Travel Medicine.* 7:89–91.

González-González AE, Aliouat-Denis CM, Carreto-Binaghi LE, Ramírez JA, Rodríguez-Arellanes G, Demanche C, Chabé M, Aliouat EM, Dei-Cas E, Taylor ML. 2012. An Hcp100 gene fragment reveals *Histoplasma capsulatum* presence in lungs of *Tadarida brasiliensis* migratory bats. *Epidemiology and Infection.* 140:1955–1963.

González-González AE, Aliouat-Denis CM, Ramírez-Bárcenas JA, Demanche C, Pottier M, Carreto-Binaghi LE, Akbar H, Derouiche S, Chabé M, Aliouat EM, Dei-Cas E, Taylor ML. 2014. *Histoplasma capsulatum* and *Pneumocystis* spp. co-infection in wild bats from Argentina, French Guyana, and Mexico. *BMC Microbiology*. 14:23.

González-González AE, Ramírez JA, Aliouat- Denis CM, Demanche C, Aliouat EN, Dei-Cas E, Chabé M, Taylor ML. 2013. Molecular detection of *Histoplasma capsulatum* in the lung of a free-ranging common noctule (*Nyctalus noctula*) from France using the Hcp100 gene. *Journal of Zoo and Wildlife Medicine.* 44(1):15–20.

Greer DL, Bolaños B. 1997. Role of bats in the ecology of *Paracoccidioides brasiliensis*: the survival of *Paracoccidioides brasiliensis* in the intestinal tract of frugivorous bat, *Artibeus lituratus*. *Sabouraudia.* 15(3):273–282.

Greer DL, McMurray DN. 1981a. Pathogenesis and immune response to *Paracoccidioides brasiliensis* in the fructivorous bat, *Artibeus lituratus*. *Sabouraudia.*19(3):165–178.

Greer DL, McMurray DN. 1981b. Pathogenesis of experimental histoplasmosis in the bat, *Artibeus lituratus*. *American Journal of Tropical Medicine and Hygiene.* 30(3):653–659.

Grose E, Marinkelle CJ. 1966. Species of *Sporotrichum*, *Trichophyton* and *Microsporum* from Colombian bats. *Tropical and Geographical Medicine.* 18:260–263.

Gugnani HC, Muotoe-Okafor FA, Kaufman L, Dupont B. 1994. A natural focus of *Histoplasma capsulatum var. duboisii* is a bat cave. *Mycopathologia*. 127(3):151–157.

Johnson LJAN, Miller AN, McCleery RA, McClanahan R, Kath JA, Lueschow S, Porras-Alfaro A. 2013. Psychrophilic and psychrotolerant fungi on bats and the presence of *Geomyces* spp. on bat wings prior to the arrival of white nose syndrome. *Applied and Environmental Microbiology* 79(18):5465–5471.

Jülg B, Elias J, Zahn A, Köppen S, Becker-Gaab C, Bogner JR. 2008. Bat-associated histoplasmosis can be transmitted at entrances of bat caves and not only inside the caves. *Journal of Travel Medicine.* 15(2):133–136.

Kajihiro ES. 1965. Occurrence of dermatophytes in fresh bat guano. *Applied Microbiology.* 13(5):720–724.

Khan ZU, Randhawa HS, Lulla M. 1982. Isolation of *Blastomyces dermatitidis* from the lungs of a bat, *Rhinopoma hardwickei hardwickei* Gray, in Delhi. *Sabouraudia.* 20(2):137–144.

Klite PD. 1965. Isolation of *Histoplasma capsulatum* from bats of El Salvador. *American Journal of Tropical Medicine and Hygiene.* 14(5):787–788.

Klite PD, Diercks FH. 1965. *Histoplasma capsulatum* in fecal contents and organs of bats in the Canal Zone. *American Journal of Tropical Medicine and Hygiene.* 14(3):433–439.

Kokurewicz T, Ogórek R, Pusz W, Matkowski K. 2016. Bats increase the number of cultivable airborne fungi in the "Nietoperek" bat reserve in western Poland. *Microbial Ecology.* 72(1):36–48.

Lorch JM, Minnis AM, Meteyer CU, Redell JA, White JP, Kaarakka HM, Muller LK, Lindner DL, Verant ML, Shearn-Bochsler V, Blehert DS. 2015. The fungus *Trichophyton redellii* sp. Nov. causes skin infections that resemble white-nose syndrome of hibernating bats. *Journal of Wildlife Diseases*. 51(1):36–47.

Lyon GM, Bravo AV, Espino A, Lindsley DL, Gutierrez RE, Rodriguez I, Corella A, Carrillo F, McNeil MM. Warnock D, Hajjeh RA. 2004. Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica, 1998–1999. *American Journal of Tropical Medicine and Hygiene.* 70(4):438–442.

Man B, Wang H, Xiang X, Wang R, Yun Y, Gong L. 2015. Phylogenetic diversity of culturable fungi in the Heshang Cave, central China. *Frontiers of Microbiology.* 6:1158.

McMurray DN, Greer DL. 1979. Immune responses in bats following intranasal infection with *Histoplasma capsulatum*. *American Journal of Tropical Medicine and Hygiene.* 28(6):1036–1039.

McMurray DN, Stroud J, Murphy JJ, Carlomagno MA, Greer DL. 1982. Role of immunoglobulin classes in experimental histoplasmosis in bats. *Developmental and Comparative Immunology.* 6(3):557–567.

McMurray DN, Thomas ME, Greer DL, Tolentino AL. 1978. Humoral and cell-mediated immunity to *Histoplasma capsulatum* during experimental infection in neotropical bats (*Artibeus lituratus*). *American Journal of Tropical Medicine and Hygiene.* 27(4):815–821.

Mok WY, Luizao LCC, Da Silva MDSB. 1982. Isolation of fungi from bats of the Amazon Basin. *Applied and Environmental Microbiology.* 44(3):570–575.

Oyeka CA. 1994. Isolation of *Candida* species from bats in Nigeria. *Mycoses.* 37(9–10):353–355.

Randhawa HS, Chaturvedi VP, Kini S, Khan ZU. 1985. *Blastomyces dermatitidis* in bats: first report of its isolation from the liver of *Rhinopoma hardwickei hardwickei* Gray. *Sabouraudia.* 23(1):69–76.

Raymond JT, White MR, Kilbane TP, Janovitz EB. 1997. Pulmonary blastomycosis in an Indian fruit bat (*Pteropus giganteus*). *Journal of Veterinarian Diagnostic Investigations.* 9:85–87.

Reiss NR, Mok WY. 1979. *Wangiella dermatitidis* isolated from bats in Manaus Brazil. *Sabouraudia.* 17(3):213–218.

Shacklette MH, Diercks FH, Gale NB. 1962. *Histoplasma capsulatum* recovered from bat tissues. *Science.* 135:1135.

Shacklette MH, Hasenclever HF. 1969. Variation of rates of natural infection with *Histoplasma capsulatum* in bats. *American Journal of Tropical Medicine and Hygiene.* 18(1):53–57.

Sugita T, Kikuchi K, Makimura K, Urata K, Someya T, Kamei K, Niimi M, Uehara Y. 2005. *Trichosporon* species isolated from guano samples obtained from bat-inhabited caves in Japan. *Applied and Environmental Microbiology.* 71(11):7626–7629.

Taylor ML, Chávez-Tapia CB, Rojas-Martínez A, Reyes-Montes MDR, del Valle MB, Zúñiga G. 2005. Geographical distribution of genetic polymorphism of the pathogen *Histoplasma capsulatum* isolated from infected bats, captured in a central zone of Mexico. *FEMS Immunology and Medical Microbiology.* 45:451–458.

Taylor ML, Chávez-Tapia CB, Vargas-Yañez R, Rodríguez-Arellanes G, Peña-Sandoval GR, Toriello C, Pérez A, Reyes-Montes MR. 1999. Environmental conditions favoring bat infection with *Histoplasma capsulatum* in Mexican shelters. *American Journal of Tropical Medicine and Hygiene.* 61(6):914–919.

Taylor ML, Hernández-García L, Estrada-Bárcenas D, Salas-Lizana R, Zancopé-Oliveira RM, De La Cruz SG, Galvão-Dias MA, Curiel-Quesada E, Canteros CE, Bojórquez-Torres G, Bogard-Fuentes CA, Zamora-Tehozol E. 2012. Genetic diversity of *Histoplasma capsulatum* isolated from infected bats randomly captured in Mexico, Brazil, and Argentina, using the polymorphism of (GA)n microsatellite and its flanking regions. *Fungal Biology.* 116:308e317.

Teixeira M, Patané JS, Taylor ML, Gómez BL, Theodoro RC, de Hoog S, Engelthaler DM, Zancopé-Oliveira RM, Felipe MS, Barker BM. 2016. Worldwide phylogenetic distributions and population dynamics of the genus *Histoplasma*. *PLoS Neglected Tropical Diseases.* 10(6):e0004732.

Tesh RB, Arata AA, Schneidau JD Jr. 1968. Histoplasmosis in Colombian bats with a consideration of some of the factors influencing the prevalence of natural infection in Chioptera. *The American Journal of Tropical Medicine and Hygiene.* 17(1):102–106.

VI

ZOONOTIC DISEASE TRANSMISSION AND BATS

15

ZOONOTIC TRANSMISSION OF DISEASE BY BATS AND OTHER ANIMALS

15.1 INTRODUCTION

The diversity of viruses and other microbes known to have zoonotic potential is increasing rapidly, with an average of three to four new human pathogens being identified each year (Woolhouse *et al.* 2012). Some of the more relevant factors influencing the likelihood of an animal species to serve as reservoirs or vectors of zoonotic disease include: (1) whether the potential hosts' geographical distribution includes the maximum area in which the microbial species is located; (2) the abundance of reservoir species in the environment; (3) the extent of interactions between the microbe and reservoir host; (4) the lack of significant illness in reservoir host along with prolonged periods of infection that permit transmission among hosts and allow microbial persistence; and (5) the habits of host and reservoir populations in both natural and anthropic environments. A tendency for the purported reservoir host to enter human dwellings or for humans to enter into reservoir hosts dwelling sites increases the chances of close contact between the reservoir species and humans that enables interspecies transmission of the microbial agent (de Oliveira *et al.* 2014). Routes of transmission include direct contact with animals or animal material (such as anthrax transmitted by combing sheep wool), animal bites and scratches; bites or other contact with arthropod vectors; and consumption of contaminated food or water (Chikeka & Dumler 2015).

Important microbial factors that influence zoonotic events include: (1) infectious dose; (2) host and vector population density; (3) biological and environmental features, such as microbial genetics and pathogenesis and climate change; (4) reassortment or recombination in multiple hosts; (5) pathogens that infect species which harbor multiple

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closely related microbes; (6) multiple routes of transmission or indirect transmission; (7) anthropogenic practices such as land use, travel, and intensified crop and animal production practices; (8) use or abuse of antimicrobial compounds; (9) loss of biodiversity; and (10) efficacy of public health systems (Asokan *et al.* 2016).

Zoonotic disease emergence can be divided into two stages: (1) "spillover" transmission of microbes from animals to humans; and (2) "stuttering transmission" that occurs when limited human-to-human infections occur, resulting in self-limiting chains of transmission, as was the case with Ebola hemorrhagic fever prior to 2014 and the initial stages of the MERS epidemic (Lo Iacono *et al.* 2016). Prior to spillover, the microbe exists solely in the animal reservoir host. Following stuttering transmission, a pathogen may adapt to human hosts in such a manner as to allow continuing transmission in humans without need for further zoonotic events, such is the case with the 2014–2016 outbreak of Ebola in West Africa and the recent ability of MERS-CoV to pass between humans in healthcare centers. In the case of human-to-human transmission via the oral–fecal route following zoonotic transmission, the microbe must adapt not only to the human host but also to harsh environmental conditions it encounters in the time period spent between humans (de Graaf *et al.* 2017). In the case of contact transmission via skin, genital mucosa, or respiratory mucosa, the pathogen factors favoring emergence of sustained human-to-human transmission following a zoonotic event are immune evasion, high viral load, and low infectious dose, while human factors include crowding, promiscuity, and the presence of co-infections (Richard *et al.* 2017).

Lo Iacono *et al*. (2016) developed a mathematical framework to unify the spillover and stuttering transmission stages, based on generalizations of Poisson processes and taking into account past human infections. They describe a number of non-biological factors that contribute to apparent transition between these two stages, including reporting bias. After quantifying pathogen dynamics in the reservoir host with information about mechanisms of contact, this framework may be useful in estimating the likelihood of spillover events. This ambitious framework needs to be tested over the course of time to determine its efficacy in a variety of different types of zoonotic transmission that involve different types of microbes which differ in rates of mutation and in different human socioeconomic settings, especially in the case of shifting human populations resulting from civil unrest and movement of large numbers of refugees from developing nations into developed areas of the world in which the majority of the population are susceptible. Even if this framework proves to be only accurate in limited conditions, such as the emergence of disease from RNA viruses in a given population with a given set of cultural and economic norms, it would still be very useful and could possibly be adjusted for utility in other situations with other groups of microbes in different human populations.

In order to better understand potential zoonotic transmission, a multifactorial approach is needed. This approach could include the fields of mathematical ecology and epidemiology; social sciences, particularly anthropology; environmental science and modelling; public health and its science–policy perspectives; and wildlife conservation organizations (Wood *et al.* 2012). Such a multifactorial approach is the goal of the "One Health" network that emphasizes the interdependence of the health of humans, animals, and ecosystems (One Health Global Network 2017). It is critical, however, that such programs do not restrict their attention to only a few groups of animals, such as bats and rodents, since other groups of animals are important either as disease reservoirs, as

amplifying or bridging hosts, or vectors that transmit the disease to humans. Part of this chapter is devoted to a few of the many groups of animals that serve in these capacities. Ignoring the roles of these animals, especially domestic animals, severely decreases the value of integrated approaches. Arthropod vectors of disease need to also be monitored since they play critical roles in directly transmitting microbes into humans.

15.2 ZOONOTIC TRANSMISSION OF INFECTION BY BATS

There have been many studies which suggest that bats have traits that give them unique potential to act as reservoir hosts for zoonotic viruses (reviewed by Calisher *et al.* 2006; Luis *et al.* 2013). Host traits associated with higher zoonotic potential include long lifespans which may allow persistence of long-term chronic infections in the host population; larger body size; prolonged torpor which slows viral replication and reduces immune system activity; flight and long-distance migration which may increase microbial dispersion; and roosting characteristics of some bat species which include very large and dense colonies that may house multiple species, allowing for ease of interspecies microbial transmission (reviewed by Luis *et al.* 2013). It should be noted that, given the high degree of bat diversity, each of the above traits is not found in all bat species. For example, some bat species are small, have solidary roosts, do not migrate, or have very little contact with humans. Additionally, migration of bats was not found to predict increased zoonotic transmission of viruses (Luis *et al.* 2013).

Some species of bats display other criteria believed to be important for functioning as zoonotic reservoir hosts, one of which is the lack of significant illness in infected animals. Persistent microbial infection of bats may result from reduced inflammatory responses that minimize immunopathology (Plowright *et al.* 2016). Bats extensively utilize interferons in viral control rather than relying heavily on the inflammatory, cell-mediated mechanisms that predominate in human anti-viral immunity. It is thus possible that at least some bat species may be better equipped to rapidly control viral replication while avoiding the pathology seen in other animal groups. Additionally, direct evidence for persistent henipavirus or filovirus infection in bats is lacking, despite extensive research efforts to prove otherwise (Plowright *et al.* 2016). Existing data concerning protective immunity against viral infection in bats does, however, indicate that immune responses differ among bat and viral species, cautioning against broad, sweeping generalizations of bat–viral interactions. Anti-bacterial, -fungal, and -protozoan responses also vary among bat and microbial species. Additionally, it has been suggested that lyssaviruses are the only virus group that cause serious disease in bats. However, other viral groups, as discussed elsewhere in this book, also cause severe or fatal infections in bats. Orthoreoviruses have been found to cause disease in the digestive, respiratory, and urinary systems of some infected bats. Symptoms include hemorrhagic enteritis, non-suppurative interstitial pneumonia, follicular hyperplasia of the spleen, and glomerulopathy (Kohl *et al.* 2012). Kaeng Khoi virus, an orthobunyavirus, has been detected in brains of dead bats. Material from the brains of these dead bats, but not normal bats, causes severe encephalitis in mice (Neill 1985; Osborne *et al.* 2003).

Moratelli and Calisher (2015) propose that based on currently available evidence, we cannot confidently link bats with emerging viruses, except for rabies. In most cases, the sole available evidence is the detection of the same or similar viruses in bats and

humans in the same locale from whence the disease had emerged. This may merely indicate that bats are similar enough to humans to allow them to temporarily host the virus, which may be present in other vertebrates or invertebrates as well. They suggest that the connections between bats, bat viruses, and human diseases are more speculative than evidence-based, but that further studies will prove that bats are competent hosts for at least some zoonotic viruses.

15.2.1 Direct or indirect zoonotic transmission by bats to humans

Much of the attention of recent emerging disease studies has focused on the role of bats as reservoir hosts of zoonotic viruses, including Marburg and Ebola filoviruses, henipaviruses, rabies virus and other lyssaviruses, and SARS-CoV and MERS-CoV coronaviruses, as described elsewhere in this book and by others (Wong *et al.* 2007; Calisher *et al.* 2008; Mühldorfer 2013; Smith & Wang 2013; Banyard *et al.* 2014; Dietrich *et al.* 2015; Wang & Cowled 2015). Routes of infection are varied and include humans being bitten or scratched by bats; consuming or inhaling aerosolized material contaminated by bat guano, urine, or saliva; or contact with bats or bat blood while hunting, butchering, preparing, or consuming bats as food. Transmission may include intermediate hosts, such as domestic animals or arthropods and other microbial vectors (reviewed by Han *et al.* 2015).

Kohl and Kurth (2014) sound a cautionary note by reminding those in the health community that, while in many cases bats have been demonstrated to carry viral and bacterial genomic sequences similar to those found in human pathogens, finding a microbe in bats similar to one in humans does not necessarily mean that zoonotic transmission from bats had occurred. In the case of viruses, such as SARS-like CoV, a few key differences in its host-binding proteins are critical in determining host cell tropism and host species range, thus even though microbes may be similar at the nucleic acid level, mere similarity is not sufficient to determine the propensity of a microbe to infect humans or, if zoonotic transmission does occur, to predict its virulence in a different host species. In the case of those lyssaviruses that are found in a number of species of European bats, infection in humans typically is fatal, so preventing infection is an important public health issue. It is also important, however, to balance risk of infection with the number of fatal human cases, especially given the endangered status of European bats. The risk of human infection with lyssaviruses in Europe is low and may be lowered still more with appropriate education (Kohl & Kurth 2014).

15.2.2 Transmission and persistence of viruses within and among bat species over large geographical ranges

Some bats have a wide geographical range, aggregate in huge colonies, migrate long distances, and may exist in close contact with humans, as is the case for *E. helvum*, found throughout Africa. Multiple genetic and serological markers indicate that this bat species forms a panmixia across the African continental range at a greater geographical scale than is known for any other mammal (Peel *et al.* 2013). This could contribute to bat-borne viral spread over a very large region and play a role in viral persistence and transmission over long distances.

A database of publications from 1934 to 2011 was used in an analysis of global viral sharing networks in bats and rodents. These networks connect hosts by their shared viruses, whether shared directly or via an intermediate host. The bat networks appear to be better connected than rodent networks in terms of number of viruses per host species, number of hosts per virus, mean degree, and connectance (Luis *et al.* 2015). This suggests that viruses may pass more readily among different bat species than between rodents, especially for gregarious bats roosting in large colonies or sharing their roosts with other bat species (Cui *et al.* 2012; Luis *et al.* 2015). Rodents have a lesser tendency to form large colonies than some bat species, whose roosts may harbor over 1 million bats. Sympatry is another very important factor in both bat and rodent networks. Regional bat migration aids in viral spread throughout the bat viral sharing network as well as increases the geographical area over which potential transmission to members of other animal orders may occur (Luis *et al.* 2015). This analysis did not explore viral transmission between Chiropterans and other orders of animals. The findings are also open to change since more viral species are being continually found. Additionally, differences in the numbers of publications focusing on bats and rodents as well as differences in testing procedures over this span of years may introduce bias.

15.2.3 Seasonal changes contributing to zoonotic transmission from bats

Spillover of viruses from bats to humans or domestic animals often shows temporal correlation, with pulses of viral excretion occurring within bat populations. Several hypotheses have been put forward to explain the underlying mechanisms of the pulses in bats (Plowright *et al.* 2016). First, the pulses may be due to seasonal epidemic cycles resulting from variations in bat population density and differences in the degree of intraspecies contacts. Bat-to-bat contacts increase during the mating period and while females are in maternity roosts. This scenario relies upon transmission of short-lived infections providing long-lasting immunity. Viral prevalence may periodically decrease in one locale, but could persist over a broad geographical range, allowing viruses to be reintroduced by migration. New outbreaks may occur as birthing cycles and waning maternal immunity increase the pool of susceptible bats. Secondly, viral pulses may result from waning immunity within bat populations, resulting in oscillating herd immunity. This was formerly seen in smallpox outbreaks in human populations. Thirdly, episodic pulses may result from periodic viral shedding by chronically infected bats due to physiological or ecological factors, including decreased immunity during pregnancy and natural or man-made environmental stressors, leading to viral reactivation. This scenario relies upon host ability to control acute infection without complete viral clearance (Plowright *et al.* 2016). Stress activates production and release of cortisol, an immunosuppressive agent that may allow reactivation of latent infection. Seasonal patterns of male aggregation and population dynamics within a given bat species differ among populations residing in temperate Colorado, in warm temperate, and in tropical areas and may affect disease dynamics, as is the case with maintenance of rabies virus in populations of *Eptesicus fuscus* residing in different climatic zones (Hayman *et al.* 2016). These differences complicate modeling of seasonality within one bat genus and even within one species.

The seasonality of hibernation may also influence infection dynamics since this state often decreases the rate of pathogen replication rates and, consequently, disease severity in and shedding by the bat host. Hibernation may also permit some microbes to survive colder temperatures and to overwinter in temperate regions (Sulkin *et al.* 1960; Hayman *et al.* 2016).

In order to more accurately determine the yearly cycles of microbial persistence, shedding, transmission, and pathogenicity in the face of seasonal variations, longitudinal studies of colonies are needed. Even more useful data could be obtained by conducting experimental, captive, or capture–recapture studies. Manual capture may often be impractical, making use of microchips and telemetry more attractive, if possible. Transmitter size, however, prohibits using this methodology in most bat species at this time (Hayman *et al.* 2013). In one such study, an individual *E. helvum* found to have filovirus-specific antibodies survived for at least 13 months afterwards, indicating long-term infection after exposure to filoviruses (Hayman *et al.* 2010). Seropositivity, however, does not necessarily imply that the bat was actually infected.

15.3 ZOONOTIC TRANSMISSION OF INFECTION BY OTHER ANIMAL SPECIES

Rodents contain more zoonotic hosts than any other order, 244 of 2220 species (Han *et al.* 2016). They also host a high number of zoonotic viruses (68, compared with 61 in bats), which is to be expected since rodents are the most abundant group of mammals. Bats, in fact, host more zoonotic viruses per species than rodents (Luis *et al.* 2013). The greatest numbers of rodent host species are in north temperate areas of North America and Europe as well as tropical Atlantic forests of Brazil (Han *et al.* 2016). More zoonotic viruses are found in mammalian host species with large amounts of overlapping distributions with other species from the same taxonomic order. Bats species having greatest levels of zoonotic viral diversity are those with one young per litter and more yearly litters as well as long life-spans. Interspecies transmission is more widespread among bats than rodents.

Rodents, however, display some important characteristics associated with potential zoonotic reservoirs: their order is evolutionarily a more ancient order than that of bats and believed to be closer to humans; is very diverse; contains many species that possess peridomestic behaviors; and some rodents may undergo torpor, as discussed below. Rodents, therefore, also pose a serious threat for zoonotic transmission to humans (Luis *et al.* 2013).

Carnivores are found in an order that also contains many zoonotic host species (49% of 285 carnivorous species) harboring at least one of 83 zoonotic pathogens unique to the order. Ungulates also have a high potential for zoonotic transmission, especially domestic species. About 32% of wild ungulate species are zoonotic hosts (73 of 247 ungulate species) and harbor 68 unique zoonotic microbes (Han *et al.* 2016). Nearly 21% of primate species may act as zoonotic hosts (77 of 365 primate species) and harbor 63 unique zoonotic microbes. By contrast, about 9.8% of bat species serve as zoonotic hosts (108 of 1100 species) and they have a much lower number of unique zoonotic microbes (approximately 27) (Han *et al.* 2016). Hotspots of bat species known to transmit microbes to humans occur in Central and South America and Southeast Asia.

The amount of contact between humans and bats is relatively small when compared with contact to rodents, except for regions in which bats are used as bushmeat.

Bacteria are responsible for more zoonotic infections than all other pathogen groups, followed by zoonotically transmitted viruses. Carnivores contain the highest numbers of both bacterial and viral zoonotic microbes. Helminths, while not the covered in this book, have the highest number of zoonotic infections and are found most commonly in rodents. Protozoans contain a lower number of zoonotic species and are found most commonly in ungulates. Bats and primates cause lower numbers of zoonoses. Viruses are the most common zoonotic microbial group in bats. Surveillance bias needs to be taken into account since the extent of surveillance scrutiny applied to viruses of bats is likely to skew the number of bat species which function as reservoirs for zoonotic viruses (Han *et al.* 2016). Increased studies of the microbiome of other groups of animals as well as the extent and type of their interactions with humans are sorely needed in order to determine the true threat of the risk of zoonotic disease potential of bats versus other mammalian groups. Several important examples of zoonotic transmission from species other than bats are discussed below in order to stress the importance of zoonoses arising from a wide range of vertebrates, some of which abide in closer contact with humans than do bats.

15.3.1 Zoonotic transmission by rodents

Rodents are the most abundant and diverse group of mammals worldwide. They negatively impact human health and economies by directly or indirectly enabling the transmission or spread of microbes that are pathogenic for humans or our agricultural animals and as well as by crop destruction. Rodents share with bats some of the characteristics that make them attractive candidates for long-term maintenance of viral populations. Like bats, some rodent species, including mice, chipmunks, and hamsters, undergo torpor (Levesque & Tattersall 2010; Chi *et al.* 2016; Sunagawa & Takahashi 2016). Additionally, some rodent species are quite long-lived (reviewed by Luis *et al.* 2015).

Rodents have been implicated as the primary reservoir hosts or vectors of many serious human disease agents. Rodents may act indirectly by amplifying microbial populations whose members are subsequently transmitted to humans by arthropods or other disease vectors. Rodents may also serve as the primary hosts or reservoirs of human pathogens that are transmitted by contact with aerosolized urine, feces, or saliva. The microbes may be maintained for extended periods of time in rodent populations by vertical or horizontal transfer. Rapid escalation in numbers of rodents may occur during periods of mild climatic conditions that increase food availability. Increased rodent numbers have been linked to increased incidence of some human diseases (Meerburg *et al.* 2009).

15.3.1.1 Zoonotic transmission of Lyme disease: Roles of rodents and bats The Lyme disease spirochete (*Borrelia burgdorferi*) uses the blacklegged tick as its primary vector for transmission to humans in the eastern US. Rodents serve as reservoir hosts for the spirochete. The rodents also play host to and infect hematophagous immature ticks, while deer host the adult ticks and bring them into closer contact with humans. Deer numbers have increased relatively recently due to loss of predators. Deer-to-human contact is also increasing as humans and deer enter the ranges of each

other. Strategies of disease-avoidance have included application of acaricides to people's skin and clothing or to rodent nesting materials and reducing close contact between humans or human dwellings and rodents. Plans to reduce zoonotic transmission of Lyme disease via reduction of deer numbers or treatment of deer with topical acaricides, however, may lead to serious problems, such as public acceptance and implementation logistics (Eisen & Dolan 2016), as well as ecosystem disturbance. Similar attempts to control zoonotic transmission from bats by decreasing their numbers may meet with some of the same problems, but may have a much greater impact both economically and ecologically than reducing deer numbers. Additionally, instead of increasing in numbers as is the case with rodents, many bat species numbers are declining. Public perception of bats also is not as positive as that of deer and thus reduction of bats might meet with greater public support. Development of other strategies to avoid contact between humans and bats would be valuable in terms of human health and bat conservation.

15.3.1.2 Zoonotic transmission of other diseases caused by spirochetes: Roles of rodents and bats

Several *Borrelia* species of spirochetes cause potentially fatal tick-borne relapsing fever, characterized by at least two episodes of high fever, painful muscles or joints, nausea and vomiting, and headache. Later in the course of the disease, jaundice, hepatosplenomegaly, and myocarditis can occur (Chikeka & Dumler 2015). Humans are infected with *Borrelia* via the bite of soft tick vectors. The ticks are infected primarily during the course of feeding on infected rodents. In the southern US, antibodies to relapsing fever spirochetes, however, were found in *E. fuscus* bats, but could not be cultured from the bat blood. A *Pipistrellus* species bat also died from fatal borreliosis in the UK. Therefore, although bats may be infected and die from relapsing fever, they appear to be only accidental hosts.

15.3.1.3 Zoonotic transmission of hemorrhagic fever arenaviruses: Roles of rodents and bats

Lassa virus is an Old World arenavirus that is the causative agent of Lassa fever, responsible for 100 000–300 000 human infections yearly in West Africa (McCormick & Fisher-Hoch 2002). The Natal multimammate rat serves as the primary reservoir host. Seroprevalance may reach 60–80% in these rodents. Routes of transmission to humans occur by inhalation of aerosolized rodent excreta or during hunting and butchering rat for consumption (reviewed by Mylne *et al.* 2015). Interestingly, mapping studies show that while this rodent is common and widely distributed across much of Sub-Saharan Africa, Lassa fever and the causative virus appear to be confined to West Africa. This discrepancy may be partially due to under-reporting of asymptomatic cases of human infection or to differences in the behavior of the various human populations throughout the range of the rodent host, including housing conditions, social relations, and agricultural practices. A better understanding of how the reservoir populations transmit the microbe amongst themselves will aid in developing a better picture of disease risk to humans living outside of the current known range of Lassa fever virus (Mylne *et al.* 2015).

Several New World arenaviruses, found primarily in South America, cause hemorrhagic fever in humans with fatality rates of around 30%. These viruses include Junin, Machupo, Guanarito, Sabia, and Chapare viruses. These are transmitted to humans via aerosolized rodent excreta or saliva (reviewed by Beltz 2011). Although another New World arenavirus, Tacaribe virus, was first isolated from the Jamaican fruit

bat (*Artibeus jamaicensis*), bats are only transiently infected and may not act as competent arenavirus carriers (Cogswell-Hawkinson *et al.* 2012).

15.3.1.4 Zoonotic transmission of hantaviruses: Roles of rodents and bats Hantavirus pulmonary syndrome or hantavirus cardiopulmonary syndrome in the Americas have high fatality rates. They are caused by Sin Nombre and related viruses. Hantaan, Dobrava-Belgrado, and similar viruses cause hemorrhagic fever with renal syndrome in Eurasia. While at least 51 species of rodents, 7 species of bats, and 20 shrews and moles serve as reservoirs for more than 80 hantavirus species, hantaviruses are primarily transmitted to humans via inhalation of aerosolized rodent excreta. Hantavirus infection in rodents is typically subclinical and can lead to lifelong viral reservoir status. Increased rodent density is the primary factor correlating with epidemics of hantaviruses in humans (reviewed by de Oliveira *et al.* 2014). The carriers of Old World hantaviruses are *Myodes, Rattus*, and *Apodemus* rodents, while Sigmodontinae rodents serve as vectors of New World hantaviruses (reviewed by Jonsson *et al.* 2010).

The hantaviruses in bats are found in insectivorous species present throughout tropical and subtropical climates of much of the world. These viruses include the Magboi, Mouyassué, Longquan, Huangpi, and Xuan Son viruses. Since these hantaviruses differ from known hantavirus species of other animals, bats appear to be their natural hosts. However, the human pathogen, Hantaan virus, has been detected in bats from Korea and, curiously, from Brazil (De Araujo *et al.* 2012). With the possible exception of Hantaan virus, only hantaviruses from rodent species have been linked to human disease (Jonsson *et al.* 2010).

15.3.2 Zoonotic transmission by companion animals

15.3.2.1 Zoonotic transmission of bacteria by companion animals Contact, including petting and licking, between household pets increases the risk of sharing microbes. Household pets include dogs, cats, rodents, rabbits, ferrets, birds, amphibians, reptiles, and domestic pigs. Some of the microbes capable of causing disease in humans are transmitted through bites or scratches, inhalation, disposal of animal urine or feces, or via the oral–fecal route. Some pets also harbor multidrug-resistant bacteria with zoonotic potential, such as methicillin-resistant *Staphylococcus aureus* (Damborg *et al.* 2016). Cat bites often are more of a concern than dog bites since cat bites are often deeper than dog bites due to their sharper teeth. Additionally, 20–80% of cat bite wounds become infected, compared with 3–18% for dog bite wounds (Talan *et al.* 1999). Some of the infections transmitted to humans include the following: (1) cat scratch fever caused by *Bartonella* species and transmitted by cats and sometimes, dogs, and rabbits; (2) leptospirosis caused by *Leptospira* species and transmitted by dogs and sometimes rats; (3) multidrug-resistant infections caused by *Staphylococcus aureus* and transmitted by dogs and cats: (4) psittacosis caused by *Chlamydia psittaci* and transmitted by birds via inhalation; and (5) salmonellosis caused by *Salmonella* species and transmitted by reptiles and to a lesser extent by birds, rodents, cats, dogs, and fish (Talan *et al.* 1999). Of these, bats also are infected with *Leptospira interrogans* and other leptospires and several *Bartonella* species (Damborg *et al.* 2016). The close contact between humans and household pets appears, however, to make pets more important than bats in zoonotic transmission of microbial infections. Dogs and opossums in Mexico and the southern

US are also increasingly becoming found to be infected with the insect-borne protozoan *Trypanosoma cruzi* (reviewed by Beltz 2011). Some bat species are also infected with this blood parasite.

15.3.2.2 Zoonotic transmission of viruses by companion animals Our knowledge of the diversity of viruses present in human companion animals is expanding rapidly, due at least in part to increased research activity, but perhaps also due to the growth in numbers and diversity of these companion animals (Reperant *et al.* 2016) and placing into close proximity animal species that do not come into contact naturally. This was the case for imported African rodents infected with the monkeypox virus which subsequently passed to prairie dogs kept in the same American pet store and, from them, into children (reviewed by Beltz 2011). Companion animals carry at least 59 viruses that infect humans and 135 that infect animals used for food production (Reperant *et al.* 2016). The human pathogens include rabies and European bat lyssaviruses and West Nile, tick-borne encephalitis, Crimean-Congo hemorrhagic fever, Aichi, hepatitis E, and influenza A viruses.

15.3.3 Zoonotic transmission by selected agricultural animals

15.3.3.1 Transmission by pigs Pigs are known to play an important role in the spillover of several zoonotic human infections, among them, one of history's deadliest pandemics, the 1918 Spanish influenza, that resulted in at least 20–40 million deaths in a very short period of time. This influenza strain is believed to have originated in pigs in the US and was then passed into bird populations, where it underwent recombination with influenza viruses of birds, before entering human populations and undergoing further recombination with human influenza viruses. Other influenza pandemics may also involve pigs (reviewed in Beltz 2011). Ebola Reston virus, originally found in macaques from the Philippines, has also been found in pigs from that country. While infection in macaques is rapidly fatal, this virus appears to be asymptomatic in pigs, which nevertheless do shed virus, making them prime candidates for the viral reservoir host. Fortunately, although some humans caring for infected monkeys seroconverted, this Ebola species does not appear to result in disease. *Rousettus amplexicaudatus* bats are also seropositive for Ebola Reston (Smith & Wang 2013), indicating that they are also exposed to this virus. Additionally, while bats are infected with Nipah virus, pigs serve as its amplifying host and appear to allow its direct transmission to humans.

Pigs play a major role in the transmission of hepatitis E virus (HEV) in developed regions of North America and Europe, although it is transmitted person-to-person in most of the developing world via consumption of water contaminated with human fecal material. HEV infection typically results in self-limiting acute hepatitis, but may cause fulminant hepatic failure in people with underlying chronic liver disease, the elderly, and pregnant women (Doceul *et al.* 2016).

HEV genotype 3 and genotype 4 have recently become a growing cause of concern in many developed regions of the world that have better sanitation and, thus, less chance of infection passing between people. The similarities of HEV genotype 3 strains in non-travelers from the US with swine viral genotype 3 suggests, but does not prove, that pigs and wild boars may play a role in HEV transmission (Drobeniuc *et al.* 2013). Pigs and

pork consumption have been implicated in zoonotic transmission in England, France, and Japan (Teo 2010; Berto *et al.* 2012, 2013). Other animals in developed countries, however, are also infected with HEV strains closely related to those infecting humans. These animals include rats and deer in the Americas (Lack *et al.* 2012; Medrano *et al.* 2012), rabbits in France (Izopet *et al.* 2012), and shellfish in Great Britain (Crossan *et al.* 2012). Several species of bats from Central America, Africa and Europe are also infected with HEV, however, this strain is not similar to that found in humans (reviewed by Drexler *et al*. 2012). Solid organ transplantation is also an important source of infection with HEV genotype 3 (Legrand-Abravanel *et al.* 2011; Pas *et al.* 2012; Drobeniuc *et al.* 2013). While swine and deer were originally considered to be the zoonotic source of human infection in the developed world, it appears that the situation is much more complex and that some of the animals which are infected by HEV may or may not be an important source of human infection. Care needs to be taken when assigning one or two animal species as major zoonotic sources of infection before thoroughly examining the potential role of other species in transmission of the disease agent to humans.

15.3.3.2 Transmission by small ruminants Small ruminants, such as sheep and goats, are also sources of bacterial or zoonotic infections, some of them serious (reviewed by Ganter 2015). Several of the more important diseases will be mentioned here.

The bacterium *Bacillus anthracis* is the causative agent of anthrax. In its respiratory form, it is a life-threatening disease in humans. Infection may occur by inhalation of bacterial spores present in soil contaminated by sheep excrement. These spores are hardy and may remain viable in the environment for decades.

Coxiella burnetii, a very small, obligate intracellular bacterium, is the causative agent of Q fever. Acute disease in humans may include a fever of up to 40 °C, shivering, severe headache, chronic fatigue, muscle pain, anorexia, and coughing. Humans are infected by inhalation of infective aerosols from ruminants or direct contact with infected animals. A large outbreak in the Netherlands was linked to a series of abortions in large dairy goat farms.

Brucella melitensis is a bacterium that serves as the causative agent of brucellosis (Malta fever) in the Middle East, Western Asia, Africa, and South America. Human infection is primarily due to consumption of dairy products from sheep and goats or inhalation of contaminated dust. Humans may develop undulant fever, arthritis, liver damage, and have miscarriages.

Rift Valley fever is due to a viral infection indirectly transmitted to humans by mosquito bites or by contact with infected sheep and goats or their animal products. Human infection is often asymptomatic but may result in a generally mild, self-limiting form that includes fever, headache, muscle pain, nausea, and sensitivity to light. Occasionally, people develop a form of hemorrhagic fever which has a fatality rate of 0.5–2.0%. Transmission from small ruminants to humans is indirect, via several species of mosquitoes.

Scrapie is a prion disease of sheep that results in spongiform encephalopathy (development of sponge-like holes in the brain). It may be transmitted to cattle by cattle feed containing sheep offal. The cattle form of infection (“mad cow disease), but not the sheep form, may be passed to humans consuming beef. To the best of our knowledge, infection is uniformly fatal.

15.4 FACTORS THAT INCREASE THE RISK OF ZOONOTIC INFECTION BY BATS

15.4.1 Increasing urbanization of bats

Increasing contact between bats and humans has been characterized by a "push and pull" mechanism. "Push" is due to increasing human incursion into and disruption of bat territorial ranges, resulting in habitat destruction and food shortages. "Pull" provides incentive for bats to enter into regions having intensive crop and food animal breeding areas due to an abundance of food (reviewed by Han *et al.* 2015). Recent changes in bat behavior also increase risk of zoonotic transmission by urbanization as bats roost in artificial structures such as bridges and old mines, as well as homes, churches, schools, and barns. Urban habituation escalates the number and length of contact between humans and bats. Simulations suggest that the total number of infectious bats would be higher in the urban, as opposed to rural clusters (Plowright *et al.* 2011). An example of indirect and direct zoonotic transmission in urban environments is found in the case of the human pathogen *Leptospira interrogans*, present in the kidneys of bats roosting in schools and houses. Rodents are the primary reservoir for zoonotic transmission and infected animals continue to shed leptospires throughout the rest of their lives (reviewed by Chikeka & Dumler 2015). Contact with infected bat urine, however, may allow bat-borne *Leptospira* spillover into humans directly or indirectly, via rodents that live or forage under bat roosts (reviewed by Dietrich *et al.* 2015).

Similarly, under natural conditions, frugivorous and nectivorous bats rely on food sources that are irregular, ephemeral, and patchily distributed in native forests, necessitating long migrations in order to maintain a continuous food supply in forested areas. Responses to the loss of their native forest habitats along the eastern coast of Australia have led flying foxes infected with Hendra virus to find alternative food sources in areas such as urban gardens whose plants provide abundant, reliable food that is available year-round, negating the need for energy-intensive, long-distance foraging and migration activities. Such appears to be the case in Hendra virus outbreaks, in which nine of the fourteen known outbreaks were near urbanized or sedentary flying fox populations in Australia (Plowright *et al.* 2011). Decreases in migration also reduce bat colonies' herd immunity and produce more intense outbreaks after reintroduction of microbes into bat groups containing many immunologically naïve animals.

15.4.2 Human activities that increase contact with bats, including the bushmeat trade

A survey in Ghana, West Africa, indicates that human activities may result in frequent contact between humans and bats in rural areas as well as urban centers. Contact occurred by several routes, including regular visitation of bat caves (46.6% of respondents; $n = 1274$); exposure via bat bites, scratches, or urine (37.4%); or consumption of bat meat (45.6%) (Anti *et al.* 2015). One community had had a hunting festival on Wednesday evenings which involved capturing bats as they returned to their roosts. Some caves serve as spiritual sanctuaries in Ghana, while other caves serve as major water sources. Cultural beliefs can increase human contact with bats. In Ghana, some believe that stirring milk with a bat's head on a stick will bring good luck (Kamins *et al.* 2015).

Trade in bat bushmeat is a common cause of human interaction with bats in Ghana (Kamins *et al.* 2011). Consumption of bats is common in many areas of Africa and Asia and, in some localities, bats are considered to be a luxury item. Roasted, fried, or smoked fruit bats are often sold in rural marketplaces. Unlike the situation in Asia, live bats are not typically found in these markets nor were they eaten raw (Anti *et al.* 2015; Kamins *et al.* 2015).

While both women and hunters perform the majority of butchering, marketplace vendors are primarily women. Only 23% of those involved in the bat-bushmeat trade perceived a significant risk of acquiring disease from bats. Education is an important factor in the bushmeat trade since education level was inversely correlated with hunting, selling, preparing, or consuming bats (Kamins *et al.* 2015). An educational presentation concerning possible risk of acquiring bat-borne disease as well as the positive economic and ecological contributions of bats to human welfare significantly improved participants' understanding of these topics, but only 55% of those interviewed said that they no longer wished to participate in the bat-bushmeat trade (Kamins *et al.* 2015).

Laws and regulations may only partially affect bat hunting in Ghana since many hunters did not know the actual dates of the bat hunting season (Kamins *et al.* 2015). Additionally, while 77% of surveyed hunters stated that they would stop hunting and selling bats if they were to receive a small fine, others said that no such actions would stop them from trafficking in bat bushmeat. Kamins *et al*. (2015) suggest that it may not be simple to minimalize risks of zoonotic spillover via the bushmeat trade since bat hunting is highly seasonal and can be started and stopped at will, while working with agricultural animals requires continuous commitment on a year-long daily activity. Nipah virus, present in Southeast Asia, may also be found in Cameroon in western Central Africa, where relatively high numbers of seropositive bats and very low numbers of humans are found in the apparent absence of human disease. Antibody-positive humans are almost exclusively people who butcher bats, particularly if residing in deforested regions. It should be noted that these African Nipah viruses only share 70% nucleotide similarity with the corresponding Asian viruses in their attachment and fusion proteins and that cross-reactive antibodies are found among henipaviruses (Pernet *et al.* 2014).

Another factor to consider in the bat-bushmeat trade is that hunting may increase prevalence of infection as well as the size of epidemic peaks, because killing of the adults may shift populations to younger, more susceptible individuals (Hayman *et al.* 2013).

15.5 STRATEGIES TO PREVENT ZOONOTIC TRANSMISSION FROM BATS TO HUMANS OR OTHER ANIMALS

Due to the great diversity existing among the various families and genera of bats, strategies designed to predict and reduce zoonotic spillover need to also differ greatly among bat groups. A better understanding of bat population ecology and temporal variations in immunological responsiveness will aid in developing successful strategic responses to the risk of human infection. This is true not only for bats, but for other animal species that are predicted to contribute to zoonotic infection. Some disease prevention strategies now in use, such as dispersing or culling bats, might be ineffective or counterproductive

if stress is a major factor in the reactivation of a given viral infection. Dispersing bat colonies may also be counterproductive if it increases the geographical range for some bat-borne viral species (Plowright *et al.* 2016).

Limited knowledge of inter-relations of pathogens and their reservoir hosts poses challenges for prevention and control of zoonotic infections (Plowright *et al.* 2016). Furthermore, a one-size-fits-all approach is inappropriate when dealing with diverse groups of animals, such as bats and rodents, which differ in terms of diet, roosting preferences, and colony size, along with other factors. Strategies aimed at predicting and preventing spillover into humans must also take into account the great diversity in microbial agents, even within a group, such as viruses. Many of the recent strategies used to combat transmission of Zika virus to humans by reducing contact between humans and the mosquito vectors used the same failed methods as were used to prevent the spread of West Nile virus in the US. The extensive differences in mosquito habitat, dietary preferences, and biting habits, in addition to potentially critical differences in human immune responses to these different viral species, need to be taken into account when implementing measures designed to prevent mosquito-borne illness. An increased understanding of factors driving microbial infection of animals is critical and must be conducted on a case-by-case basis which considers both potential vector and reservoir hosts and the specific microbe involved. Increased understanding of host–microbe interactions is also important in conservation efforts since bats and other animal species themselves often fall prey to viral, bacterial, fungal, and parasitic diseases. Some of these microbes may be transmitted to bats as a result of human activity, as is the case of white-nose syndrome.

15.6 CONCLUSIONS

The search for viruses with zoonotic potential carries the risk of becoming a self-fulfilling prophecy in which limiting pathogen testing to pathogens considered to be of public health importance will of necessity limit the results to those pathogens (Reperant *et al.* 2016). In a similar vein, restricting the search of animal species that may serve as reservoir hosts or vectors of microbes with zoonotic potential also skews the results and may serve to artificially amplify the true risk of spillover from those animal species. This may indeed be the case with bats, since some global health strategies have focused intensively on the zoonotic potential of bats' virome and have accordingly found many viruses that infect various bat species as well as humans. Intensive scrutiny of microbes present in other orders of animals would undoubtedly also detect many microbes with zoonotic potential. It is critical to differentiate between the presence of a microbe in an animal order or species and the actual degree of likelihood of those animals being a key to zoonotic transmission.

Microbial infection and persistence within populations is multifactorial, including infection of host cells and individual hosts, in addition to the species population and community structure. Microbial persistence within a given population requires the ability to enter, survive and replicate within, and exit from the hosts as well as the means and opportunity to transit between individual hosts. Survival within an individual requires the ability of microbes to evade host immune responses. Periods of stress in animals lowers their immunity since stress hormones, such as cortisol, are immunosuppressive

and may increase shedding by bat populations as well as increasing their susceptibility to infection. A study of the effects of roost disturbance in Australian flying foxes, however, found no significant differences in Hendra virus shedding or urinary cortisol concentrations (Edson *et al.* 2015).

While genomic sequences as well as antibodies against various microbes may be found within mammalian populations, the responsible microbes may not be recovered and may be able to experimentally infect the presumptive host species only rarely or not at all, as is the case with Ebola virus in bats (Plowright *et al.* 2015). Low levels of microbial shedding by the proposed reservoir species would hinder intra- and interspecies transmission. The population-dense, three-dimensional roost structures of bats, however, may aid in microbial transmission through droplets or aerosols of microbes excreted in urine, feces, or via the respiratory route. Over time, continuous exposure of individual bats to microbes may lead to a high probability of infection. Microbes with short infectious periods in hosts having low movement rates may result in patchy microbial dynamics within populations, while long infectious periods and high movement rates may lead to more homogenized dynamics. Migratory bat species, thus, appear to be more likely to distribute microbes across bat populations and communities. Viral shedding in many bat species often occurs in discrete seasonal pulses, often linked to decreases in immunity during pregnancy and loss of maternal antibodies in juveniles, and may support short infectious periods with microbial extinction, followed by recolonization between roosts or intermittent shedding from chronically infected individuals (Plowright *et al.* 2015).

Spillover requires the presence of infected reservoir and recipient hosts in a given region to reach a critical density. Growth in human population sizes and movement into new regions, combined with changes in land usage, increases interaction between humans and bats, rodents, and other animal populations, as does movement of bats and rodents into semi-urban or urban areas in search of food or shelter as their habitat is refigured by human activity. In the case of indirect transmission, microbes must be able to survive in the surrounding environment long enough to reach a susceptible recipient host, which needs to be exposed to the microbe in sufficient quantities to establish infection. Temperature, humidity, and pH all affect the length of microbial survival in the environment (Plowright *et al.* 2015). In the case of spill-over into humans, those microbes which are able to be passed through populations via a human-to-human route would be more likely to establish an ongoing chain of transmission that is particularly dangerous to immunocompromised individuals even if the resulting infections are typically asymptomatic or mild. Periodic, severe outbreaks in many immunocompetent individuals may result if the infection is highly pathogenic. In the latter case, the microbes do not persist in humans long-term since susceptible hosts are either killed or quarantined or herd immunity reaches a critical level. In this case, outbreaks may reappear sporadically due to rare spill-over events reintroducing the microbe into now susceptible human populations, leading to bursts of time in which morbidity and mortality rates are high, as was the case in the 2014–2015 outbreak of Ebola hemorrhagic fever in West Africa and the periodic epidemics of plague or pandemic influenza. Due to the rarity of such instances, spill-over events are hard to predict, even with intensive active surveillance of potential reservoir hosts.

Governmental bodies and health organizations often tend to react hastily in the face of perceived emerging infections, without studying potentially disastrous consequences

to complex and delicate ecological systems. The tendency of public media to sensualize news about the risk of zoonotic disease may cause a backlash against some animal species, even without evidence supporting the utility of such actions. Major efforts to monitor potential reservoir hosts for the presence of microbes may be misguided since many groups of animals are infected by scores of microbes that are genetically similar to those which infect humans, yet due to their differences from human pathogens, these newly detected microbes may not be able to actually infect humans or cause anything more than mild disease in most people.

The development of predictive models has the possibility of specifically identifying spatiotemporal factors involved in zoonotic transmission and permit appropriate protective actions to be taken (Hayman *et al.* 2013). Current models, however, may not be accurate or useful in the face of incomplete data, the multitude of currently unknown variables, and constantly changing environmental, sociological, and economic conditions. The difficulties in developing accurate models, even within one bat population at one point in the yearly reproductive cycle may be illustrated by examining the paradoxical increase and decrease in risk of developing microbial disease during lactation. The increased contact between mother and infant during lactation increases the risk of microbial transmission to the young bats, however the presence of maternal antibodies in the milk decreases risk of productive infection. Colony density and changes in density occurring during migration, cycling between summer maternity roosts and winter hibernacula, maternal health, behavioral differences between male and female bats, and the numbers and strains of circulating microbes, in addition to the propensity of multi-segmented RNA viruses to mutate and undergo reassortment, are other factors that confound modeling (Hayman *et al.* 2013). Host-relatedness and overlap in geographical ranges also affect interspecies transmission. There is also a need to examine the effect of bat infection with multiple microbes, pathogenic or nonpathogenic, as is often found in animals or humans in their natural environments. In humans, infection by some viruses results in a temporary state of reduced immunity to other microbes.

Massive programs to eliminate either the reservoir or vector hosts are likely to have not only very large economic costs, but also devastating environmental impact. For example, if broad-spectrum insecticides are used to prevent rare human infections, valuable insectivorous animal species, including bats and birds, may also be severely harmed by alteration of the food chain and the consumption of insecticides which may become concentrated in the insectivores' fat tissue (Thies *et al.* 1996; Brinati *et al.* 2016). Since massive vaccination campaigns are too expensive and impractical to maintain in the case of rare zoonotic events, perhaps our limited national and international funds could best be utilized to develop broad-spectrum antimicrobial drugs to counter the inevitable zoonotic epidemics.

REFERENCES

Anti P, Owusu M, Agbenyega O, Annan A, Badu EK, Nkrumah EE, Tschapka M, Oppong S, Adu-Sarkodie Y, Drosten C. 2015. Human–bat interactions in rural West Africa. *Emerging Infectious Diseases.* 21(8):1412–1421.

Asokan JV, Asokan V. 2016. Bradford Hill's criteria, emerging zoonoses, and One Health. *Journal of Epidemiology and Global Health.* 6:125–129.

Banyard AC, Evans JS, Luo TR, Fooks AR. 2014. Lyssaviruses and bats: emergence and zoonotic threat. *Viruses.* 6:2974–2990.

Beltz LA. 2011. *Foundations of Emerging Infectious Diseases: A Guide to Diseases, Causative Agents, and Surveillance*. Jossey-Bass and APHA Press: San Francisco, CA.

Berto A, Grierson S, Hakze-van der Honing R, Martelli F, Johne R, Reetz J, Ulrich RG, Pavio N, Van der Poel WHM, Banks M. 2013. Hepatitis E virus in pork liver sausage, France. *Emerging Infectious Diseases.* 19(2):264–266.

Berto A, Martelli F, Grierson S, Banks M. 2012. Hepatitis E virus in pork food chain, United Kingdom, 2009–2010. *Emerging Infectious Diseases.* 18(8):1358–1360.

Brinati A, Oliveira J, Oliveira VS, Barros MS, Carvalho BM, Oliveira LS, Queiroz MEL, Matta SLP, Freitas MB. 2016. Low, chronic exposure to endosulfan induces bioaccumulation and decreased carcass total fatty acids in neotropical fruit bats. *Bulletin of Environmental Contamination and Toxicology.* 97:626–631.

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. 2006. Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews.* 19(3):531–545.

Calisher CH, Holmes KV, Dominguez SR, Schountz T, Cryan P. 2008. Bats prove to be rich reservoirs for emerging viruses. *Microbe.* 3:521–528.

Chi QS, Wan XR, Geiser F, Wang DH. 2016. Fasting-induced daily torpor in desert hamsters (*Phodopus roborovskii*). *Compative Biochemistry and Physiology. Part A: Molecular Integrative Physiology.* 199:71–77.

Chikeka I, Dumler JS. 2015. Neglected bacterial zoonoses. *Clinical Microbiology and Infection.* 21:404–415.

Cogswell-Hawkinson A, Bowen R, James S, Gardiner D, Calisher CH, Adams R, Schountz T. 2012. Tacaribe virus causes fatal infection of an ostensible reservoir host, the Jamaican fruit bat. *Journal of Virology.* 86(10):5791–5799.

Crossan C, Baker PJ, Craft J, Takeuchi Y, Dalton HR, Scobie L. 2012. Hepatitis E virus genotype 3 in shellfish, United Kingdom. *Emerging Infectious Diseases.* 18(12):2085–2087.

Cui J, Tachedjian M, Wang L, Tachedjian G, Wang LF, Zhang, S. 2012. Discovery of retroviral homologs in bats: implications for the origin of mammalian gammaretroviruses. *Journal of Virology.* 86:4288–4293.

Damborg P, Broens EM, Chomel BB, Guenther S, Pasmans F, Wagenaar JA, Weese JS, Wieler LH, Windahl U, Vanrompay D, Guardabassi L. 2016. Bacterial zoonoses transmitted by household pets: State-of-the-art and future perspectives for targeted research and policy actions. *Journal of Comparative Pathology.* 155:S27–S40.

De Araujo J, Thomazelli LM, Henriques DA, Lautenschalager D, Ometto T, Dutra LM, Aires CC, Favorito S, Durigon EL. 2012. Detection of hantavirus in bats from remaining rain forest in São Paulo, Brazil. *BMC Research Notes.* 5:690.

de Graaf M, Beck R, Caccio SM, Duim B, Fraaij PL, Le Guyader FS, Lecuit M, Le Pendu J, de Wit E, Schultsz C. 2017. Sustained fecal-oral human-to-human transmission following a zoonotic event. *Current Opinion in Virology.* 22:1–6.

de Oliveira RC, Guterres A, Fernandes J, D'Andrea PS, Bonvicino CR, de Lemos ERS. 2014. Hantavirus reservoirs: Current status with an emphasis on data from Brazil. *Viruses.* 6:1929–1973.

Dietrich M, Mühldorfer K, Tortosa P, Markotter W. 2015. *Leptospira* and bats: Story of an emerging friendship. *PLoS Pathogy.* 11(11):e1005176.

Doceul V, Bagdassarian E, Demange A, Pavio N. 2016. Zoonotic hepatitis E virus: classification, animal reservoirs and transmission routes. *Viruses* 8:270.

Drexler JF, Seelen A, Corman VM, Tateno AF, Cottontail V, Zerbinati RM, Gloza-Rausch F, Klose SM, Adu-Sarkodie Y, Oppong SK, Kalko EK, Osterman A, Rasche A, Adam A, Müller MA,

Ulrich RG, Leroy EM, Lukashev AN, Drosten C. 2012. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. *Journal of Virology.* 86:9134–9147.

Drobeniuc J, Greene-Montfort T, Le N-T, Mixson-Hayden TR, Ganova-Raeva L, Dong C, Novak RT, Sharapov UM, Tohme RA, Teshale E, Kamili S, Teo C-G. 2013. Laboratory-based surveillance for hepatitis E virus infection, United States, 2005–2012. *Emerging Infectious Diseases.* 19(2):218–222.

Edson D, Field H, McMichael L, Jordan D, Kung N, Mayer D, Smith C. 2015. Flying-fox roost disturbance and Hendra virus spillover risk. *PLoS ONE.* 10(5):e0125881.

Eisen L, Dolan MC. 2016. Evidence for personal protective measures to reduce human contact with blacklegged ticks and for environmentally based control methods to suppress host-seeking blacklegged ticks and reduce infection with Lyme disease spirochetes in tick vectors and rodent reservoirs. *Journal of Medical Entomology*. pii: tjw103.

Ganter M. 2015. Zoonotic risks from small ruminants. *Veterinary Microbiology.* 181:53–65.

Han BA, Kramer AM, Drake JM. 2016. Global patterns of zoonotic disease in mammals. *Trends in Parasitology.* 32(7):565–577.

Han H-J, Wen H-l, Zhou C-M, Chen F-F, Luo L-M, Liu J-w, Yu X-J. 2015. Bats as reservoirs of severe emerging infectious diseases. *Virus Research.* 205:1–6.

Hayman DTS, Bowen RA, Cryan PM, McCracken GF, O'Shea TJ, Peel AJ, Gilbert A, Webb CT, Wood JLN. 2013. Ecology of zoonotic infectious diseases in bats: current knowledge and future directions. *Zoonoses and Public Health.* 60:2–21.

Hayman DTS, Emmerich P, Yu M, Wang L-F, Suu-Ire R, Fooks RL, Cunningham AA, Wood JLN. 2010. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS ONE.* 5(8):e11978.

Hayman DTS, Fooks AR, Marston DA, Garcia-R JC. 2016. The global phylogeography of lyssaviruses – challenging the 'out of Africa' hypothesis. *PLoS Neglected Tropical Diseases.* 10(12):e0005266.

Izopet J, Dubois M, Bertagnoli S, Lhomme S, Marchandeau S, Boucher S, Kamar N, Abravanel F, Guérin J-L. 2012. Hepatitis E virus strains in rabbits and evidence of a closely related strain in humans, France. *Emerging Infectious Diseases.* 18(8):1274–1281.

Jonsson CB, Figueiredo LTM, Vapalahti O. 2010. A global perspective on hantavirus ecology, epidemiology, and disease. *Clinical Microbiology Reviews.* 23(2):412–441.

Kamins AO, Restif O, Ntiamoa-Baidu Y, Suu-Ire R, Hayman DT, Cunningham AA, Wood JL, Rowcliffe JM. 2011. Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. *Biological Conservation.* 144(12):3000–3008.

Kamins AO, Rowcliffe JM, Ntiamoa-Baidu Y, Cunningham AA, Wood JLN, Restif O. 2015. Characteristics and risk perceptions of Ghanaians potentially exposed to bat-borne zoonoses through bushmeat. *EcoHealth.* 12:104–120.

Kohl C, Kurth A. 2014. European bats as carriers of viruses with zoonotic potential. *Viruses.* 6:3110–3128.

Kohl C, Lesnik R, Brinkmann A, Ebinger A, Radonic A, Nitsche A, Muhldorfer K, Wibbelt G, Kurth A. 2012. Isolation and characterization of three mammalian orthoreoviruses from European bats. *PLoS One.* 7:e43106.

Lack JB, Volk K, Van Den Bussche RA. 2012. Hepatitis E virus genotype 3 in wild rats, United States. *Emerging Infectious Diseases.* 18(8):1268–1273.

Legrand-Abravanel F, Kamar N, Sandres-Saune K, Lhomme S, Mansuy J-M, Muscari F, Sallusto F, Rostaing L, Izopet J. 2011. Hepatitis E virus infection without reactivation in solid-organ transplant recipients, France. *Emerging Infectious Diseases.* 17(1):30–37.

Levesque DL, Tattersall GJ. 2010. Seasonal torpor and normothermic energy metabolism in the Eastern chipmunk (*Tamias striatus*). *Journal of Comparative Physiology B.* 180(2):279–292.

Lo Iacono GL, Cunningham II, Fichet-Calvet E, Garry RF, Grant DS, Leach M, Moses LM, Nichols G, Schieffelin JS, Shaffer JG, Webb CT, Wood JLN. 2016. A unified framework for the infection dynamics of zoonotic spillover and spread. *PLoS Neglected Tropical Diseases.* 10(9):e0004957.

Luis AD, Hayman DTS, O'Shea TJ, Cryan PM, Gilbert AT, Pulliam JRC, Mills JN, Timonin ME, Willis CKR, Cunningham AA, Fooks AR, Rupprecht CE, Wood JLN, Webb CT. 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special*? Proceedings of the Royal Society B.* 280:20122753.

Luis AD, O'Shea TJ, Hayman DTS, Wood JLN, Cunningham AA, Gilbert AT, Mills JN, Webb CT. 2015. Network analysis of host–virus communities in bats and rodents reveals determinants of cross-species transmission. *Ecology Letters.* 18:1153–1162.

McCormick JB, Fisher-Hoch SP. 2002. Lassa fever. *Current Topics in Microbiology and Immunology.* 262:75–109.

Medrano C, Boadella M, Barrios H, Cantú A, García Z, de la Fuente J, Gortazar C. 2012. Zoonotic pathogens among white-tailed deer, northern Mexico, 2004–2009. *Emerging Infectious Diseases.* 18(8):1372–1374.

Meerburg BG, Singleton GR, Kijlstra A. 2009. Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology.* 35(3):221–270.

Moratelli R, Calisher CH. 2015. Bats and zoonotic viruses: can we confidently link bats with emerging deadly viruses? *Memórias do Instituto Oswaldo Cruz.* 110(1):1–22.

Mühldorfer K. 2013. Bats and bacterial pathogens: a review. *Zoonoses and Public Health.* 60:93–103.

Mylne AQN, Pigott DM, Longbottom J, Shearer F, Duda KA, Messina JP, Weiss DJ, Moyes CL, Golding N, Hay SI. 2015. Mapping the zoonotic niche of Lassa fever in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 109:483–492.

Neill WA. 1985. Kaeng Khoi. In: *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*, 3rd edition. N Karabatsos (ed.). American Society for Tropical Medicine and Hygiene: San Antonio, TX, pp. 533–534.

One Health Global Network. 2017. http://www.onehealthglobal.net. Accessed January 7, 2017.

Osborne JC, Rupprecht CE, Olson JG, Ksiazek TG, Rollin PE, Niezgoda M, Goldsmith CS, An US, Nichol ST. 2003. Isolation of Kaeng Khoi virus from dead *Chaerephon plicata* bats in Cambodia. *Journal of General Virology.* 84(Pt 10):2685–2689.

Pas SD, de Man RA, Mulders C, Balk AHMM, van Hal PTW, Weimar W, Koopmans MPG, Osterhaus ADME, van der Eijk AA. 2012. Hepatitis E virus infection among solid organ transplant recipients, the Netherlands. *Emerging Infectious Diseases.* 18(5):869–872.

Peel AJ, Sargan DR, Baker KS, Hayman DTS, Barr JA, Crameri G, Suu-Ire R, Broder CC, Lembo T, Wang L-F, Fooks AR, Rossiter SJ, Wood JLN, Cunningham AA. 2013. Transmission and persistence of viruses within and among bat species over large geographical ranges. *Nature Communications.* 4:2770.

Pernet O, Schneider BS, Beaty SM, LeBreton M, Yun TE, Park A, Zachariah TT, Bowden TA, Hitchens P, Ramirez CM, Daszak P, Mazet J, Freiberg AN, Wolfe ND, Lee B. 2014. Evidence for henipavirus spillover into human populations in Africa. *Nature Communications*. DOI: 10.1038/ncomms6342.

Plowright RK, Eby P, Hudson PJ, Smith IL, Westcott D, Bryden WL, Middleton D, Reid PA, McFarlane RA, Martin G, Tabor GM, Skerratt LF, Anderson DL, Crameri G, Quammen D, Jordan D, Freeman P, Wang L-F, Epstein JH, Marsh GA, Kung NY, McCallum H. 2015.

Ecological dynamics of emerging bat virus spillover. *Proceedings of the Royal Society B*. 282: 2014–2124.

Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, Daszak P. 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proceedings from the Royal Society B*. 278:3703–3712.

Plowright RK, Peel AJ, Streicker DG, Gilbert AT, McCallum H, Wood J, Baker ML, Restif O. 2016. Transmission or within-host dynamics driving pulses of zoonotic viruses in reservoir–host populations. *PLoS Neglected Tropical Diseases.* 10(8):e0004796.

Reperant LA, Brown IH, Haenen OL, de Jong MD, Osterhaus ADME, Papa A, Rimstad E, Valarcher J-F, Kuiken T. 2016. Companion animals as a source of viruses for human beings and food production animals. *Journal of Comparative Pathology.* 155:S41–S53.

Richard M, Knauf S, Lawrence P, Mather AE, Munster VJ, Müller MA, Smith D, Kuiken T. 2017. Factors determining human-to-human transmissibility of zoonotic pathogens via contact. *Current Opinion in Virology.* 22:7–12.

Smith I, Wang L-F. 2013. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Current Opinion in Virology.* 3:84–91.

Sulkin SE, Allen R, Sims R, Krutzsch PH, Kim C. 1960. Studies on the pathogenesis of rabies in insectivorous bats II. Influence of environmental temperature. *Journal of Experimental Medicine.* 112:595–617.

Sunagawa GA, Takahashi M. 2016. Hypometabolism during daily torpor in mice is dominated by reduction in the sensitivity of the thermoregulatory system. *Scientific Reports.* 6:37011.

Talan DA, Citron DM, Abrahamian FM, Moran GJ, Goldstein EJ. 1999. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. *New England Journal of Medicine.* 340:85–92.

Teo CG. 2010. Much meat, much malady: changing perceptions of the epidemiology of hepatitis E. *Clinical Microbiology and Infection.* 16:24–32.

Thies ML, Thies K, McBee K. 1996. Organochlorine pesticide accumulation and genotoxicity in Mexican free-tailed bats from Oklahoma and New Mexico. *Archives of Environmental Contamination and Toxicology.* 30(2):178–187.

Wang L-F, Cowled C (eds). 2015. *Bats and Viruses: A New Frontier in Emerging Infectious Diseases*. Wiley Blackwell: Hoboken, NJ.

Wong S, Lau S, Woo P, Yuen KY. 2007. Bats as a continuing source of emerging infections in humans. *Reviews of Medical Virology.* 17:67–91.

Wood JLN, Leach M, Waldman L, MacGregor H, Fooks AR, Jones KE, Restif O, Dechmann D, Hayman DTS, Baker KS, Peel AJ, Kamins AO, Fahr J, Ntiamoa-Baidu Y, Suu-Ire R, Breiman R, Epstein IH, Field HE, Cunningham AA. 2012. A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical Transactions of the Royal Society B: Biological Sciences.* 367:2881–2892.

Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M. 2012. Human viruses: discovery and emergence. *Philosophical Transactions of the Royal Society B: Biological Sciences.* 367:2864–2871.

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