Chapter 19
Virology and Immunology of Bats

Tony Schountz

Abstract  Bats harbor many pathogens of veterinary and human health concern, including several emerging and reemerging viruses such as lyssaviruses, filoviruses, henipaviruses, and SARS-like coronaviruses. Despite immune responses to these viruses, many bats remain infected without disease and likely shed virus to other bats and mammals. Little is known about bat immune systems or how the immune responses of bats control infections. The recent characterization of genome and transcriptome sequences of several bat species suggests they are similar to other mammals. These data indicate that bats possess orthologous genes, antibodies, and cells involved in innate and adaptive immune responses as do other mammals, but bats likely evolved unique mechanisms for controlling viruses that cause disease in other species. It is unclear how these diseases affect bat ecology, and thus, a greater understanding of immunology and infection is needed to understand health impact on bats.

19.1 Introduction

In recent years a number of viruses that cause substantial human and veterinary disease have been detected in or isolated from bats (Calisher et al. 2006). The economic impact of these viruses has been billions of dollars and these diseases have had social and medical impacts (Field 2009). Many viruses persist without pathology in bat populations, whereas some cause diseases. While much is known about the diseases in humans and livestock, virtually nothing is known about bats or how bat immune systems control viral infections.
Most viruses infect one or a few principal reservoir host species, often with little or no disease (Calisher et al. 2006). Because viruses are dependent upon their hosts for replication, they often limit pathology. The apathogenic virus–reservoir relationship is one of the coadaptations with each becoming biochemically and genetically optimized to allow virus replication without host disease. If hosts become immunocompromised, the balance of this relationship is altered, sometimes compromising both host and virus. Disease usually occurs when viruses inadvertently infect another susceptible species, termed spillover, and biochemical processes are no longer optimized between the virus and the new host species.

19.1.1 Reservoirs and Virus Ecology

There are three broad outcomes when a virus is introduced: (1) no infection occurs because the virus cannot utilize the biochemical machinery of the animal; (2) infection that may or may not cause disease, followed by an immune response that may or may not prevent disease; or (3) an apathogenic infection that allows the virus to persist. Oftentimes, viruses have evolved infectious mechanisms that render them innocuous in their reservoir host species that may account for the lack of an immune response, a result of coevolutionary adaptations. However, some viruses may cause disease in some members of the reservoir species depending on the age, genetics, or immune status of the animal. Other viruses replicate slowly and can take months or years to manifest disease, yet healthy-appearing animals can transmit virus to others prior to disease onset.

19.1.2 Immune Evasion, Persistence, and Pathogenesis

Vertebrate cells possess antiviral pathways and proteins that interfere with viral infections. However, many viruses have evolved countermeasures that provide a competitive advantage. Countermeasures have only been studied in a few animal models (e.g., mice, ferrets, or nonhuman primates); however, these countermeasures have evolved in the reservoir hosts, not in spillover or experimental organisms. How they function, qualitatively or quantitatively, in the reservoir may be substantially different than how they function in a spillover or experimental host where disease is often the outcome.

19.1.3 Infectious Diseases of Bats

Microbes play an important role in the health of all vertebrates. Some microbes, termed normal flora, provide nutrients in the gastrointestinal tract and help defend
against other pathogenic microbes. A bat pathogen, Tacaribe virus, was discovered during a rabies surveillance program near Port of Spain, Trinidad and Tobago (Downs et al. 1963). Many Jamaican (Artibeus jamaicensis) and great fruit-eating bats (Artibeus lituratus) were found dead or dying from suspected rabies. However, pathological assessment failed to detect rabies virus and subsequent work led to the isolation of Tacaribe virus from 11 bats. Recent experimental infection of A. jamaicensis with Tacaribe virus caused a disease in some bats that is similar to human South American hemorrhagic fevers, including neurological manifestations, and the virus was cleared by other bats (Cogswell-Hawkinson et al. 2012). Ebolavirus-like viral RNA was detected in many dead insectivorous Schreiber’s bats (Miniopterus schreibersii) collected in Spain, Portugal, and France (Negredo et al. 2011). More recently, several pathogenic microbes were detected in organs of European bats collected post-mortem, including pneumonia and myocarditis associated with members of several bacterial families and a protozoan and gastrointestinal disease associated with Pasteurella multocida and Yersinia pseudotuberculosis, trematode infestation, nematodes, and coccidiosis (Muhldorfer et al. 2010, 2011a, b, c). It is unclear if these microbes caused the diseases or were opportunistic pathogens; the difficulties of conducting experimental infections on bats to test Koch’s postulates has hampered disease etiology research.

19.2 The Immune Systems of Bats

19.2.1 Overview of the Immune Response

The vertebrate immune response is responsible for containment of infections using a variety of cells (Table 19.1) that operate in a highly orchestrated manner. For many infectious agents, this results in the clearance of the agent. However, others persist for lengthy periods, sometimes the life of the animal. Should the immune response fail to contain the infection, disease often occurs that compromises the host’s health leading to its death. In other instances, an aggressive immune response can cause immunopathology that can also lead to death. The balance of the immune response must limit pathogen-induced disease without substantial immunopathology while containing the infection.

The immune response occurs in two principal phases. The innate response is initiated by preexisting molecules and cells that recognize products common to infectious agents. The adaptive response, which takes several days to begin, provides highly specific long-term immunity and uses two groups of lymphocytes, B cells that produce antibodies, some of which can neutralize viruses, and T cells that coordinate activities of cells during immune responses and kill cells harboring pathogens.

Currently, the study of bat immune responses suffers from a lack of immunological reagents reactive to bat proteins, the tremendous species diversity of bats (Baker et al. 2013b), and because few captive colonies are available for experimental manipulation. Because bats are often protected by laws, bats cannot be euthanized
| Cell                        | Distribution        | Major functions                                                                                                                                 |
|-----------------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Neutrophil                  | Blood               | Infiltrates tissues during innate phase and induces inflammation and granulocyte release                                                        |
| Basophil                    | Blood               | During innate phase produces heparin and histamine to mediate inflammation; abundant during ectoparasite infections                              |
| Eosinophil                  | Blood               | Granulocytic cells that induce inflammation and secrete inflammatory cytokines and reactive oxygen species; abundant during helminth infections |
| Mast cell                   | Blood               | Binds to IgE antibodies in tissues and secretes histamine and heparin during inflammatory responses; may be derived from basophils                  |
| Monocyte                    | Blood               | Infiltrates tissues and differentiates into tissue macrophages                                                                                   |
| Macrophage                  | Most tissues        | Resident phagocytic cells that participate in innate and adaptive immune responses; antigen presentation to helper T cells                        |
| Natural killer (NK) cell    | Blood               | Infiltrates tissues during the innate phase and kills cells that are infected with viruses                                                        |
| Myeloid dendritic cell (mDC)| Most tissues        | Migrates with antigen to regional lymph nodes to stimulate adaptive immune responses of T and B cells; secretes IL-12                              |
| Plasmacytoid dendritic cell (pDC) | Most tissues | Migrates with antigen to regional lymph nodes to stimulate adaptive immune responses of T and B cells; secretes IFN-α |
| Follicular dendritic cell (FDC) | Secondary lymphoid tissues | Presents protein antigens in their native confirmation to B cells to stimulate antibody production                                               |
| Helper T (Th) cell (lymphocyte) | Secondary lymphoid tissues | Secretes cytokines during the adaptive phase that modulate local immune responses; provides help to B cells for class switching and affinity maturation and to CTL for sustained antiviral responses |
| Cytotoxic T cell (CTL) (lymphocyte) | Infected tissues/lymphoid tissues | During the adaptive phase, recognizes and kills cells infected with pathogens (e.g., viruses, some bacteria)                                        |
| Regulatory T cell (Treg) (lymphocyte) | Lymphoid tissues | Suppresses inflammatory immune responses to mitigate immunopathology                                                                         |
| B cell                      | Secondary lymphoid tissues | Synthesizes and secretes antibodies that bind to antigens and neutralize activity and/or facilitate phagocytosis by other cells, such as macrophages. Requires Th cells for class switching to other immunoglobulin classes and production of high affinity antibodies |

Adapted from Abbas et al. 2010. Cell Mol Immunol. 6th Ed. Saunders-Elsevier Press
for microbe surveys, for collection of tissues, or for experimental infections. Fortunately, new technologies are emerging that should facilitate understanding of how bats respond to infections.

19.2.2 Innate Immune Mechanisms

Groups of microbes have many molecular structures in common. Gram-negative bacteria have lipopolysaccharide and many viruses synthesize RNA in the cytoplasm of a cell. Vertebrates have evolved pattern recognition receptors (PRR) that bind these common microbial motifs for detection of infectious threats. The two broad PRR systems in vertebrate cells are the Toll-like receptors (TLR) and RIG-like helicases (RLH) and have been identified in Pteropus alecto, Rousettus leschenaultii, and A. jamaicensis using genetic and biochemical analyses (Cowled et al. 2011, 2012; Iha et al. 2010; Papenfuss et al. 2012; Shaw et al. 2012).

Type I interferon (IFN-α, IFN-β) response in mammals is triggered by TLR and RLH signaling and is mediated by more than a 100 proteins, and genes encoding proteins in these pathways have been identified in several bat species (Kepler et al. 2010; Omatsu et al. 2008; Papenfuss et al. 2012; Shaw et al. 2012). When activated by IFN-α or IFN-β, the cell enters an antiviral state and attenuates many of the cellular biochemical pathways needed by viruses for replication, including protein and nucleotide synthesis. It also leads to increased expression of major histocompatibility complex (MHC) proteins, which are essential for T cell responses and transition to the adaptive phase of the immune response. Type III IFNs (IFN-λ) are encoded by up to three genes in mammals (Ifnl1, Ifnl2, Ifnl3) and also play roles in antiviral immune responses, although their functions are less well characterized. Nonetheless, type III interferons were identified in several bat species (Zhou et al. 2011) and likely are important in viral infection management.

19.2.3 Immune System Cells and Tissues

Development of immune cells occurs in primary lymphoid tissues, which include the bone marrow, where most immune cell development begins, and the thymus, the principal site of T cell maturation. Secondary lymphoid tissues are where microbial antigens are processed and presented to B cells for antibody production and to T cells for activation of various functions. The spleen is a secondary lymphoid organ responsible for controlling blood-borne infections, whereas the lymph nodes control infections of nearby tissues. All tissues have lymphatic vessels providing a conduit for antigen-presenting cells (APCs) and draining lymph fluid to the lymph nodes that act as filters and depots for concentration of antigens and APCs. During infections, the antigen-specific T and B lymphocytes in the lymph nodes have cognate interactions with each other and with APCs, leading to clonal expansion of
antigen-specific lymphocytes and activation of the adaptive immune response. Antibodies are produced by the B cells in these lymph nodes and enter the lymphatic vessels or the blood for rapid distribution. Tertiary lymphoid tissues are typically small collections of immune cells distributed within solid organs and are involved in organ-specific infections and can traffic to the regional lymph nodes to participate in T cell and B cell activation.

Several subsets of T cells occur in mammals that have specific activities. Cytotoxic T lymphocytes (CTL) express the cell surface cluster of differentiation (CD) glycoprotein CD8. These cells recognize infected cells and kill them, thereby depriving the virus of the resources necessary for replication. Helper T (Th) cells express the surface glycoprotein CD4 and contribute by secreting cytokines that mediate local immune responses. Another subset of T cells is the regulatory T (Treg) cell that tempers the immune response and controls inflammation. In many infectious diseases, one of these Th cell types is associated with resistance or susceptibility to disease.

Little work has been conducted on bat lymphocytes, but the presence of high-titer IgG during immune responses demonstrates both B cells, which secrete immunoglobulins, and Th cells, which direct class switching and affinity maturation as an immune response evolves, are found in bats. The presence of B and T cells in Pteropus giganteus (surface immunoglobulins and cells sensitive to T cell mitogens) has been described (Chakraborty and Chakravarty 1983, 1984; Chakravarty and Sarkar 1994; Paul and Chakravarty 1987; Sarkar and Chakravarty 1991). However, virtually no work has been conducted to examine recall T cell functions of bats in response to antigens.

Cells resembling follicular dendritic cells (FDC), which present antigens in their native conformations to B cells, occur in P. giganteus (Sarkar and Chakravarty 1991) and are distinct from dendritic cells, which present peptide antigens to Th cells as part of the transition from innate to adaptive immunity. Reports of these and other cell types in bats are limited; although bats have MHC class II antigens for presenting peptide antigens to helper T cells (Mayer and Brunner 2007; Schad et al. 2011), no work on MHC class I molecules for cytotoxic T cell antigen presentation has been described even though bat transcriptomes reveal their presence (Papenfuss et al. 2012; Shaw et al. 2012). It is likely that bat immune systems are largely similar to other mammals in regard to cell types and functions.

19.2.4 Immunoglobulins

Five major classes of immunoglobulins are found in mammals: IgM, IgG, IgA, IgE, and IgD, and bats examined also have some or all of these (Butler et al. 2011). IgG, IgM, IgA, and IgE are secreted by B cells; IgD is typically a membrane-bound surface immunoglobulin receptor that is not secreted. These bivalent antibodies bind to the antigens by noncovalent interactions and have several activities that impair the
microbes’ abilities to sustain infection, including neutralization of viruses or toxins, or by marking the antigen for destruction by phagocytic cells or complement proteins that are ever present in blood.

IgM is the first antibody produced during infection and has low affinity because it cannot undergo affinity maturation in the B cells that synthesize it. However, because it is a pentamer (five IgM molecules covalently linked by a J chain) and has a valency of ten, it has high avidity and interacts with up to ten antigens, leading to complex aggregates that facilitate immune responses. Within days to weeks, IgG antibodies appear in the blood and the IgM response typically wanes. Importantly, during an immune response many of these antigen-specific IgM-secreting B cells undergo two critical events: class switching to IgG, IgA, or IgE, and affinity maturation that leads to antibodies with such high affinity that they effectively bind to antigen irreversibly under physiologic conditions. While IgG is found in the blood, lymph, and tissues, IgA is secreted into the mucosal tissues and is effective in combating infectious agents in those sites. In other mammals, both class switching and affinity maturation are driven by T cells; thus, the occurrence of high-titer antibody of these classes implies T cell participation in bat antibody responses.

The evolution of antibody genes has been examined in only a few bat species. In agreement with other mammals, the divergence of IgG appears to have occurred after speciation. *Myotis lucifugus* has five IgG subclasses, *Eptesicus fuscus* has two IgG subclasses, *Carollia perspicillata* appears to have a single IgG, and *Cynopterus sphinx* has three IgG subclasses (Butler et al. 2011). The biological functions of these subclasses have yet to be determined. IgM, IgE, and IgA have been detected as well; however, IgD was detected in *M. lucifugus* and *E. fuscus* but not in *C. perspicillata* or *C. sphinx*. It is unclear if IgD is truly absent in these species or if the employed cloning strategies were unsuccessful.

A closer scrutiny of *M. lucifugus* immunoglobulin variable gene segments shows substantial diversity of variable-heavy (V_H) germline gene segments (Bratsch et al. 2011). The somatic mutation rate among these genes appears lower than other mammals; however, the species has many more joining (J_H) and diversity (D_H) segments, suggesting it may rely on combinatorial and junctional diversity and less on somatic hypermutation for antibody diversity.

### 19.2.5 Complement

The complement (C’) system is composed of proteins having specificity and enzymatic activities for controlling microbial infections. Complement activity occurs in *E. fuscus, M. lucifugus, Tadarida brasiliensis,* and *Pteropus vampyrus* and many of the functions are similar to other mammals. However, in *E. fuscus* the activity appears less sensitive to cold, perhaps reflecting a need for immunological activity during hibernation or torpor (Allen et al. 2009; Hatten et al. 1973).
19.2.6 Cytokines

Cytokines are a large group of hormone-like proteins essential for immune system development and activities. They are biologically active in picomolar concentrations and provide important noncognate signaling during immune responses. More than 100 cytokines and chemokines have been described in mammals and it is evident that bats have orthologs for most, if not all, of these genes.

Cytokines bind to specific receptors found on certain cells and induce signal transduction that leads to gene expression or repression. The secretion of cytokines is typically brief and without storage because of their potency and potential for inducing immunopathology. They are expressed early in the innate phase through the end of the adaptive phase and activate cells of the immune response for tissue repair after clearance of the microbe.

Many cytokines have been described in bat species (Cogswell-Hawkinson et al. 2011; Iha et al. 2009; Janardhana et al. 2012) and searches of the genomic and transcriptome databases (e.g., GenBank, NCBI Trace Archive, etc.) (Papenfuss et al. 2012; Shaw et al. 2012) reveal many others. Most analysis has been phylogenetic and a significant limitation for evaluating these molecules is the lack of specific reagents for their detection, such as monoclonal antibodies. However, recent advances in gene expression analysis should provide valuable information on the roles of these molecules during viral infections.

19.3 Viruses of Bats

More than 100 viruses from many families have been isolated from or detected in bats (Calisher et al. 2006) (Table 19.2) with few known to be transmitted to humans or other animals. Some cause severe diseases, including rabies virus and other lyssaviruses, ebolaviruses and marburgvirus, severe acute respiratory syndrome (SARS) coronavirus-like viruses, and Nipah and Hendra viruses. Novel herpesviruses were recently detected in bats and other viruses that cause zoonotic diseases have also been detected; however, it is unknown if bats are reservoirs or if infections were incidental.

19.3.1 Rabies Virus and Lyssaviruses

More than 50,000 people die each year from rabies, most in Africa or Asia (Banyard et al. 2011) and most are transmitted by dog bites (see Chap. 18). Persons at risk of rabies exposure, including bat biologists and veterinarians, should be immunized and have their antibody titers checked. An exposure event in a vaccinated person should be followed by booster immunizations to minimize the risk of developing rabies, which is nearly always fatal.
Rabies virus and other lyssaviruses belong to the family *Rhabdoviridae* that are widely distributed and infect vertebrates, invertebrates, and plants. The genus *Lyssavirus* has many species that infect bats, including rabies virus, Lagos bat virus, Australian lyssavirus, and two European bat lyssaviruses, and although distinct viruses, all subsequent diseases are termed rabies because of the nearly identical pathology (Johnson et al. 2011). Rabies likely existed in the Americas prior to European colonization, with reports of Spanish conquistadors dying after vampire bat bites (Blanton et al. 2011). A concerted rabies control program instituted by the Pan American Health Organization has dramatically reduced rabies cases in urban regions (Belotto et al. 2005). Dog bites accounted for 65 % of rabies transmission to humans, whereas bats accounted for 14.7 %. By immunizing dogs, the number of human and canine rabies cases dropped by 90 %. However, because of immunizing companion animals and other wildlife, bats have become an important reservoir for rabies virus. Hematophagous bats, particularly *Desmodus rotundus*, are an important vector for transmitting rabies virus to livestock because of encroachment on bat habitat (Banyard et al. 2011).

Rabies-like existed in the Americas prior to European colonization, with reports of Spanish conquistadors dying after vampire bat bites (Blanton et al. 2011). A concerted rabies control program instituted by the Pan American Health Organization has dramatically reduced rabies cases in urban regions (Belotto et al. 2005). Dog bites accounted for 65 % of rabies transmission to humans, whereas bats accounted for 14.7 %. By immunizing dogs, the number of human and canine rabies cases dropped by 90 %. However, because of immunizing companion animals and other wildlife, bats have become an important reservoir for rabies virus. Hematophagous bats, particularly *Desmodus rotundus*, are an important vector for transmitting rabies virus to livestock because of encroachment on bat habitat (Banyard et al. 2011).

The ecology of rabies virus in bats is highly complex and poorly understood. A recent study developed and validated a model of rabies virus transmission in temperate big brown bats (*E. fuscus*) in Colorado (George et al. 2011). Seasonal mechanisms were important in the maintenance and transmission of rabies virus within bat populations. Because of the long incubation period of rabies viruses and the low mortality of bats during hibernation, infected bats survive into the next season. They then exit hibernation and become infected during the warm season. This cycle is highly seasonal and complex, and the model accurately predicted the dynamics of rabies virus transmission in this bat population.

### Table 19.2 Virus families detected in or isolated from bats

| Virus family         | Viruses |
|----------------------|---------|
| *Rhabdoviridae*      | 14      |
| *Paramyxoviridae*    | 9       |
| *Coronaviridae*      | 14      |
| *Togaviridae*        | 3       |
| *Flaviviridae*       | 19      |
| *Bunyaviridae*       | 6       |
| *Reoviridae*         | 9       |
| * Arenaviridae*      | 1       |
| *Herpesviridae*      | 12      |
| *Retroviridae*       | 1       |
| *Picornaviridae*     | 1       |
| *Papillomaviridae*   | 1       |
| *Adenoviridae*       | 7       |
| *Astroviridae*       | 1       |
| *Filoviridae*        | 3       |
| *Orthomyxoviridae*   | 1       |
| Unclassified         | 6       |

Adapted from Calisher, Childs et al. 2006; C.H. Calisher, Personal communication
season as a reservoir for transmission to other bats, particularly naive juveniles, in
the spring and summer. As juvenile mortality increased, transmission declined
within the population. Thus, the combination of long incubation of rabies virus and
lack of biochemical activity of the virus during hibernation likely contributes to
some bats remaining persistently infected for years. Because of the diversity of bat
ecology, it is likely that lyssavirus transmission and maintenance will vary substan-
tially between bat species.

19.3.2 Coronaviruses

The outbreak of SARS in Southeast Asia in 2002 was caused by a newly discovered
coronavirus, SARS-CoV. More than 8,000 cases were reported in 32 countries with
a nearly 10 % fatality rate (Field 2009). Chinese investigators initially believed the
host was either the masked palm civet (Paguma larvata) or the raccoon dog
(Nyctereutes procyonoides) because SARS-CoV-like viruses were isolated from
these animals in local live animal “wet markets” (Ksiazek et al. 2003). However,
subsequent fieldwork suggested the Chinese horseshoe bats (Rhinolophus spp.)
were more likely the original source (Lau et al. 2005; Li et al. 2005, Chap. 18). The
prevalence of antibodies to SARS-CoV was as high as 84 % in bat populations.
While the genotypes of the bat coronaviruses were distinct from the human SARS-
CoV, and thus named SARS-like coronaviruses, phylogenetic evaluation indicated
the human SARS-CoV was likely descendent from the bat virus. Since, new coro-
naviruses have been identified in other bat species. A recent outbreak of another
coronavirus disease in humans Middle East respiratory syndrome (MERS), was
associated with a virus with substantial phylogenetic similarity to coronaviruses
isolated from bats in Southeast Asia (van Boheemen et al. 2012), suggesting trans-
mission to humans is a continuing threat. The risk of contracting coronavirus infec-
tion from handling bats appears to be low; however, additional studies are needed to
fully understand the risks to bat biologists (Stockman et al. 2008).

A survey of Colorado bats identified coronavirus RNA sequences distantly
related to SARS-CoV in E. fuscus and M. occultus, but not T. brasiliensis, M. cili-
olabrum, M. evotis, Lasionycteris noctivagans, or M. volans (Dominguez et al.
2007). Two other coronavirus sequences were also detected in C. perspicillata and
Glossophaga soricina in Trinidad and Tobago (Carrington et al. 2008), while a sim-
ilar virus occurred in M. nattereri and M. daubentoni in the United Kingdom
(August et al. 2012). Coronavirus sequences were also detected in four species of
western European bats (M. daubentoni, M. dasycneme, Nyctalus noctula,
Pipistrellus pipistrellus) commonly found in urban areas (Reusken et al. 2010), rais-
ing the prospect of human or veterinary spillover. Coronavirus in the same group
as the SARS-CoV have been detected in several horseshoe bats (Rhinolophus hip-
posideros) in Slovenia, but not in six other species examined (Rihtaric et al. 2010),
and in D. rotundus in Brazil (Brandao et al. 2008). Additional coronavirus sequences
have been detected in Asia (Shirato et al. 2012; Woo et al. 2012) and there is genetic
and serological evidence of coronaviruses in Africa (Muller et al. 2007; Pfefferle et al. 2009). Thus far, no coronaviruses have been isolated from bats; only sequences and antibodies have been detected.

The use of deep sequencing was employed to identify novel coronaviruses in *E. fuscus*, *Perimyotis subflavus*, and *M. lucifugus* (Donaldson et al. 2010). In one night, 41 bats were captured and oral and fecal samples collected for RNA and DNA sequencing resulting in 76 matched coronavirus sequences.

The ecology of bat coronaviruses is unclear. However, one study (Drexler et al. 2011) followed coronavirus, astrovirus, and adenovirus transmission in a maternal colony of *Myotis myotis* in Germany for three years. Coronaviruses and astroviruses amplified within the colony during their best reproductive year, suggesting the viruses had no negative impact on reproduction. This supports the reservoir model of virus maintenance within a vertebrate host.

19.3.3 Henipaviruses

In 1994 an outbreak of an acute respiratory disease with a high fatality rate from encephalitis occurred in 14 horses and a trainer near Hendra, Australia (Field 2009). Several other small outbreaks have since occurred and a paramyxovirus, Hendra virus (HeV), was identified as the causative agent. A similar disease occurred near Nipah, Malaysia, in 1998 at a hog farm that killed hogs and ethnic Chinese abattoir workers. Of 256 cases, 105 people died and over one million hogs were euthanized, at a cost of US$500 million, to prevent further spread of the new paramyxovirus, Nipah virus. Another outbreak occurred in Bangladesh with a high number of cases and fatality rate. Together, these viruses were classified into a new genus, *Henipavirus*, and members have also been detected in Africa. Each virus has been associated with pteropid bats, including the black flying fox (*Pteropus alecto*), gray-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spectacled flying fox (*P. conspicillatus*). Subsequently, other paramyxoviruses have been discovered in bat species from other continents (Kurth et al. 2012; Sasaki et al. 2012; Wilkinson et al. 2012).

In experimental infections of black flying foxes, HeV was detected in the kidneys but none of the bats exhibited signs of disease (Halpin et al. 2011). Virus was isolated from urine and it is thought this is a principal transmission mode. Virus was also detected in the throat swabs, rectal swabs, and blood, and intranasal infection may be a means of acquiring virus.

Transmission to humans was likely a result of anthropogenic environmental changes and encroachment upon bat habitat (Field 2009). Expansion of hog farms by deforestation increased contact of fruit bats with hogs. In addition, planting of fruit orchards near hog farms caused localized habitat sinks that contributed to increased contact between bats and hogs, leading to virus spillover. Once virus entered the hog population, transmission to humans occurred, which lead to human to human transmission. Thus, agricultural intensification likely contributed to the transmission of virus to humans in the Malaysian outbreak (Pulliam et al. 2012).
Henipaviruses have also been detected in Madagascan fruit bats (Eidolon dupreanum) in Ghana (Hayman et al. 2008). Serology studies have detected antibodies that recognized Nipah and Hendra viruses and in vitro virus neutralization tests demonstrated cross-reactivity to these viruses. Henipavirus RNA from three putative species was also detected in the African straw-colored fruit bat (E. helvum) (Baker et al. 2013a). No human disease has been associated with African henipaviruses; however, considering the pathogenicity of Hendra and Nipah viruses, it is possible these viruses can cause human disease.

The ecology of Nipah virus is unclear, but it is evident that virus can persist in captive Pteropus vampyrus bats for over a year (Sohayati et al. 2011). In this study, antibody levels varied substantially among infected bats, with some maintaining high titers and others with low titers for 10 months. Maternal antibody was present in juveniles and persisted up to 14 months. Of particular concern for captive populations, some bats were seropositive, then seronegative, only to become seropositive again after many months, and virus was isolated from one of these bats. This exposes the unreliability of serology for determining the infection status of a bat that could lead to transmission of Nipah virus to unsuspecting handlers. It is prudent to assume the bats are infected even if they are seronegative.

Nipah virus V protein disrupts human type I and type II interferon responses (Rodriguez et al. 2002, 2004) by interacting with STAT1 and STAT2, two critical proteins involved in interferon signaling and transcription of many other antiviral proteins. The V protein appears to bind to one STAT1 and one STAT2 to form a trimer that presumably interferes with STAT1/STAT2 translocation into the nucleus where they normally act as transcription factors that drive the expression of many antiviral genes. However, it is unclear how the V protein behaves in Pteropus bat reservoirs where the protein has been shaped by evolutionary pressures.

19.3.4 Filoviruses

Six viruses in the family Filoviridae may be hosted by bats: Zaire ebolavirus, Taï forest ebolavirus (formerly Cote d’Ivoire ebolavirus), Sudan ebolavirus, Reston ebolavirus, Bundibugyo ebolavirus, and Lake Victoria marburgvirus. Another unclassified filovirus was also identified during an outbreak in Yambio county, southern Sudan (Onyango et al. 2007). Ebolavirus RNA has been detected in several fruit bat species, including the little collared fruit bat (Myonycteris torquata), hammer-headed fruit bat (Hypsognathus monstrosus), and Franquet’s epauletted bat (Epomops franqueti) (Leroy et al. 2005). Antibodies to ebolaviruses were also detected in other bats of these species but viral RNA was not detected in those bats, suggesting the immune responses cleared the virus or reduced viral loads to levels undetectable by PCR. A large serological survey for antibodies specific to filoviruses conducted in Gabon detected antibodies in Egyptian fruit bats, although five other bat species had a lower prevalence. RNA from an eighth ebolavirus-like virus, Lloviu virus, was detected in tissues from dead Schreiber’s bats collected in Spain,
Portugal, and France (Negredo et al. 2011). Whereas filovirus infection of fruit bats appears to be nonpathogenic, infection of the European insectivorous bats may have caused death.

Only Lake Victoria marburgvirus has been isolated in cell culture from bats (Towner et al. 2009). In 2007 an outbreak of Marburg hemorrhagic fever occurred in workers at Kitaka cave in western Uganda. A survey of the cave revealed large numbers of resident Egyptian fruit bats (Rousettus aegyptiacus) and Hipposideros species. About 5% (31/611) of the Egyptian fruit bats had marburgvirus RNA, whereas only one of 609 Hipposideros had detectable RNA. Juveniles had a higher prevalence (10.3%) compared to adults (4.2%), while pregnant bats had the lowest prevalence (2.1%). Placentas from pregnant females did not have viral RNA, making vertical transmission unlikely. All bats appeared healthy, suggesting infection had no adverse effect and supporting the hypothesis the species is a reservoir of Lake Victoria marburgvirus.

While the spillover mechanism of filoviruses to humans is unknown, an Ebola fever outbreak in 2007 was traced to consumption of bats (Leroy et al. 2009). More than 260 people were infected with 186 deaths during the outbreak. Each year in April, thousands of migrating bats settle in trees near Ndongo and Koumelele islands in the Democratic Republic of Congo. A palm oil plantation had been established in 1925 near the Lulua River which produced fruit in April and provided a source of food for the bats. Villagers hunted the bats with shotguns as a food source. By mid-May the bats left the area to continue their migration and fewer bats were hunted thereafter, and cases of Ebola disease diminished.

### 19.3.5 Other Viruses

Many other viruses have been detected in bats, including herpesviruses, adenoviruses, flaviviruses, astroviruses, influenza viruses, bunyaviruses, arenaviruses, alphaviruses, reoviruses, retroviruses, picornaviruses, and papillomaviruses (Calisher et al. 2006; Donaldson et al. 2010; Janoska et al. 2011; Watanabe et al. 2009; Wibbelt et al. 2007). These viruses have been detected in only 104 bat species; thus, more than 1,100 species have yet to be examined (C.H. Calisher, Personal communication). Until surveillance is conducted on bat populations, it is difficult to ascertain if infectious diseases have ecological consequences on bat populations.

### 19.4 Studying Bat Viruses and Host Response

#### 19.4.1 Virus Detection and Isolation

The great majority of viruses detected in bats thus far have been with serology (testing for antibodies to specific viruses) or PCR amplification. Few bat viruses have
been isolated in cell culture or in experimentally inoculated animals. While obtaining serological data or sequences from new viruses is valuable, one cannot perform experimental infection research; the virus must be isolated in an infectious state.

The use of suckling mice has led to the isolation of many viruses from other vertebrate species. This process requires the gentle homogenization of an infected tissue in a buffer, such as cell culture medium, followed by filtration or centrifugation to remove potential bacterial contamination, and inoculation into newborn mice with incompetent immune systems. Oftentimes, intracranial inoculations are required to establish infection. Inoculation of juvenile or adult rodents can be attempted; however, their immune response may clear the virus.

Cell culture is frequently useful and can yield virus free of contaminating substances that come from suckling mouse tissues. Many cell lines from invertebrates and vertebrates have been established that are susceptible to many viruses. However, two lines from the African green monkey, VERO (CCL-81), and a subline, VERO E6 (CRL-1586), are commonly used because of their susceptibility to divergent viruses. These lines are deficient in their type I interferon pathways that likely contribute to their susceptibility to a wide array of viruses. Additionally, cell lines can be established from tissues collected from bats, which may be susceptible to infection and can provide a better understanding of virus-host cell interactions. Because cell lines allow high-throughput screening and isolation, it is typically the first method of choice.

19.4.2 Antibody Detection

During infection vertebrates respond by producing antibodies specific for the agent. Initially, IgM is produced within days after infection and is followed by other isotypes, particularly IgG and IgA antibodies that undergo affinity maturation. Detection of certain isotypes from blood samples can provide clues as to how recent an infection occurred. Detection of only IgM suggests a very recent, ongoing infection, while detection of IgM and other isotypes suggests infection occurred days or weeks ago. Detection of IgG but not IgM usually signifies infection in the distant past, months to years.

The detection of IgM is often performed using a capture ELISA. This assay requires a species-specific anti-IgM antibody, usually produced in goats or rabbits. No commercial anti-bat IgM antibodies are available; thus, it is necessary for investigators to produce their own. This process typically involves immunization of other mammals (e.g., goats, rabbits, mice) with purified IgM fragments followed by purification of the anti-IgM from the serum.

Development of anti-bat IgG antibodies is simpler in most instances. Several companies manufacture protein-A/protein-G columns that have high affinity for IgG, and Bethyl Laboratories produces a goat anti-bat IgG that is reactive to antibodies from ten bat species (and likely more). Once purified, whole bat IgG can be used as an immunogen for producing antiserum for use in ELISA or other immunoassays.
Enzyme conjugates of protein-A/protein-G, such as horseradish peroxidase, are also commercially available and are often suitable for detecting virus-specific IgG in mammals (Schountz et al. 2007). These molecules have very high affinity for IgG of many mammalian species and have an incubation time of about 30 min. This permits the development of rapid field tests for serology and identification of bats that may have been infected with specific agents. This reagent must be carefully examined for reactivity to IgG for each species of interest as it varies in its affinity for antibodies.

19.4.3 Cellular Methods

The detection of antibodies specific to an agent is of limited value for assessment of immune response, but isolation of cells involved in infection and immune responses provides substantially more information. Activities such as cellular responses to infection, innate responses by immune cells, and adaptive responses by T and B cells provide qualitative and quantitative information. In particular, cell cultures can identify subsets of T cells and their cytokines that are active during immune responses, but their cultivation from outbred animals can be daunting and the lack of colonies of bats for experimental infections makes such work difficult. Nonetheless, it may be possible to cultivate virus-specific T cells provided two important cytokines are available: interleukin-2 (IL-2) for propagating T cells and granulocyte-macrophage colony-stimulating factor (GM-CSF) for propagating autologous antigen-presenting cells from the bone marrow. Each of these cytokines is cross-reactive in many species. For example, human IL-2 can be used to propagate Mus musculus T cells.

19.4.4 NextGen Sequence Analysis

Advances in genome and transcriptome sequencing have accelerated in recent years (Glenn 2011). The introduction of 454 pyrosequencing has been followed by additional high-throughput sequencing. The Illumina and SOLiD platforms have increased reads and reduction of costs has brought these technologies within reach of many projects. Other technologies, including PacBio, Ion Torrent, Ion Proton, HeliScope, and Starlight, will provide additional options for investigators involved in bat research. The principal difficulty of these technologies is the volume of data generated, approaching 600 gigabases with the Illumina HiSeq2000. Management and reduction of this amount of data requires substantial computational resources and personnel. However, these technologies will allow rapid development of expression assays, such as real-time PCR or cDNA arrays. RNA-seq will also allow a bioinformatics approach to quantifying expression of protein-coding RNAs and microRNAs that may have relevance to host responses.
19.5 Conclusion

Bats are important reservoirs of viruses; however, like other mammals, they are also susceptible to many viruses and infectious agents from other organisms, including other bats, that may have important impact on their health and ecology. The biology of bats and the historical neglect of bats and their viruses make investigation of these viruses difficult. However, new technologies and adapted existing technologies should facilitate the rapid study of host response and disease susceptibility in bat species and should lead to increased understanding of the importance of bats and their viruses, which is mutually beneficial to both bat biologists and public health scientists.

References

Allen LC, Turmelle AS, Mendonca 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). J Comp Physiol B 179(3):315–323. doi: 10.1007/s00360-008-0315-3

August TA, Mathews F, Nunn MA (2012) Alphacoronavirus detected in bats in the United Kingdom. Vector Borne Zoonotic Dis 12(6):530–533. doi: 10.1089/vbz.2011.0829

Baker KS, Todd S, Marsh GA, Cramer G, Barr J, Kamins AO, Peel AJ, Yu M, Hayman DT, Nadjm B, Mtove G, Amos B, Reyburn H, Nyarko AK, Suu-Ire R, Murcia PR, Cunningham AA, Wood JL, Wang LF (2013a) Novel potentially-zoonotic paramyxoviruses from the African straw-colored fruit bat, Eidolon helvum. J Virol 87:1348–1358. doi: 10.1128/JVI.01202-12

Baker ML, Schountz T, Wang LF (2013b) Antiviral immune responses of bats: a review. Zoonoses Public Health 1:1–13, http://www.ncbi.nlm.nih.gov/pubmed/23302292

Banyard AC, Hayman D, Johnson N, McElhinney L, Fooks AR (2011) Bats and lyssaviruses. Adv Virus Res 79:239–289. doi: 10.1016/B978-0-12-387040-7.00012-3

Belotto A, Leanes LF, Schneider MC, Tamayo H, Correa E (2005) Overview of rabies in the Americas. Virus Res 111(1):5–12. doi: 10.1016/j.virusres.2005.03.006

Blanton JD, Palmer D, Dyer J, Rupprecht CE (2011) Rabies surveillance in the United States during 2010. J Am Vet Med Assoc 239(6):773–783. doi: 10.2460/javma.239.6.773

Brandão PE, Scheffer K, Villarreal LY, Achkar S, Oliveira Rde N, Fahl Wde O, Castilho HG, Kotait I, Richtenhain LJ (2008) A coronavirus detected in the vampire bat Desmodus rotundus. Braz J Infect Dis 12(6):466–468

Bratsch S, Wertz N, Chaloner K, Kunz TH, Butler JE (2011) The little brown bat, M. lucifugus, displays a highly diverse V H, D H and J H repertoire but little evidence of somatic hypermutation. Dev Comp Immunol 35(4):421–430. doi: 10.1016/j.dci.2010.06.004

Butler JE, Wertz N, Zhao Y, Zhang S, Bao Y, Bratsch S, Kunz TH, Whitaker JO Jr, Schountz T (2011) The two suborders of chiropterans have the canonical heavy-chain immunoglobulin (Ig) gene repertoire of eutherian mammals. Dev Comp Immunol 35(3):273–284. doi: 10.1016/j.dci.2010.08.011

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006) Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 19(3):531–545. doi: 10.1128/CMR.00017-06

Carrington CV, Foster JE, Zhu HC, Zhang JX, Smith GJ, Thompson N, Auguste AJ, Ramkissoon V, Adesiyun AA, Guan Y (2008) Detection and phylogenetic analysis of group 1 coronaviruses in South American bats. Emerg Infect Dis 14(12):1890–1893. doi: 10.3201/eid1412.080642

Chakraborty AK, Chakravarty AK (1983) Plaque forming cell assay for antibody secreting cells in the bat Pteropus giganteus. Indian J Exp Biol 21(1):5–7
Chakraborty AK, Chakravarty AK (1984) Antibody-mediated immune response in the bat, *Pteropus giganteus*. Dev Comp Immunol 8(2):415–423

Chakravarty AK, Sarkar SK (1994) Immunofluorescence analysis of immunoglobulin bearing lymphocytes in the Indian fruit bat: *Pteropus giganteus*. Lymphology 27(2):97–104

Cogswell-Hawkinson AC, McGlaughlin ME, Calisher CH, Adams R, Schountz T (2011) Molecular and phylogenetic characterization of cytokine genes from Seba’s short-tailed bat (*Carollia perspicillata*). Open J Immunol 4:31–39

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. *J Virol* 86(10):5791–5799. doi: 10.1128/JVI.00201-12

Cowled C, Baker M, Tachedjian M, Zhou P, Bulach D, Wang LF (2011) Molecular characterisation of toll-like receptors in the black flying fox *Pteropus alecto*. Dev Comp Immunol 35(1):7–18. doi:10.1016/j.dci.2010.07.006

Cowled C, Baker ML, Zhou P, Tachedjian M, Wang LF (2012) Molecular characterisation of RIG-I-like helicases in the black flying fox, *Pteropus alecto*. Dev Comp Immunol 36(4):657–664. doi:10.1016/j.dci.2011.11.008

Dominguez SR, O'Shea TJ, Oko LM, Holmes KV (2007) Detection of group 1 coronaviruses in bats in North America. Emerg Infect Dis 13(9):1295–1300. doi: 10.3201/eid1309.070491

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. *J Virol* 84(24):13004–13018. doi: 10.1128/JVI.01255-10

Downs WG, Anderson CR, Spence L, Aitken THG, Greenhall AH (1963) Tacaribe virus, a new agent isolated from Artibeus bats and mosquitoes in Trinidad, West Indies. Am J Trop Med Hyg 12:640–646

Drexler JF, Corman VM, Wegner T, Tateno AF, Zerbinati RM, Gloza-Rausch F, Seebens A, Muller MA, Drosten C (2011) Amplification of emerging viruses in a bat colony. Emerg Infect Dis 17(3):449–456. doi: 10.3201/eid1703.100526

Field HE (2009) Bats and emerging zoonoses: henipaviruses and SARS. Zoonoses Public Health 56(6–7):278–284. doi:10.1111/j.1863-2378.2008.01218.x

George DB, Webb CT, Farnsworth ML, O’Shea TJ, Bowen RA, Smith DL, Stanley TR, Ellison LE, Rupprecht CE (2011) Host and viral ecology determine bat rabies seasonality and maintenance. Proc Natl Acad Sci USA 108(25):10208–10213. doi:10.1073/pnas.1010875108

Glenn TC (2011) Field guide to next-generation DNA sequencers. Mol Ecol Resour 11(5):759–769. doi:10.1111/j.1755-0998.2011.03024.x

Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, Rahaman SA, Hughes T, Smith C, Field HE, Daszak P (2011) Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. Am J Trop Med Hyg 85(5):946–951. doi:10.4269/ajtmh.2011.10-0567

Hatten BA, Lutskus JH, Sulkin SE (1973) A serologic comparison of bat complements. J Exp Zool 186(2):193–206. doi:10.1002/jez.1401860210

Hayman DT, Suu-Ire R, Breed AC, MeEachern JA, Wang L, Wood JL, Cunningham AA (2008) Evidence of henipavirus infection in West African fruit bats. PLoS One 3(7):e2739. doi:10.1371/journal.pone.0002739

Iha K, Omatsu T, Watanabe S, Ueda N, Taniguchi S, Fujii H, Ishii Y, Kyoto 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. J Vet Med Sci 71(12):1691–1695

Iha K, Omatsu T, Watanabe S, Ueda N, Taniguchi S, Fujii H, Ishii Y, Kyoto S, Akashi H, Yoshikawa Y (2010) Molecular cloning and expression analysis of bat toll-like receptors 3, 7 and 9. J Vet Med Sci 72(2):217–220

Janardhana V, Tachedjian M, Cramer G, Cowled C, Wang LF, Baker ML (2012) Cloning, expression and antiviral activity of IFN gamma from the Australian fruit bat, *Pteropus alecto*. Dev Comp Immunol 36(3):610–618. doi:10.1016/j.dci.2011.11.001

Janoska M, Vidovszky M, Molnar V, Liptovszky M, Harrach B, Benko M (2011) Novel adenoviruses and herpesviruses detected in bats. Vet J 189(1):118–121. doi:10.1016/j.tvjl.2010.06.020
Johnson N, Brookes SM, Healy DM, Spencer Y, Hicks D, Nunez A, Wells G, Fooks AR (2011) Pathology associated with a human case of rabies in the United Kingdom caused by European bat lyssavirus type-2. Intervirology 55(5):391–394. doi:10.1159/000333019

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. doi:10.1186/1471-2164-11-444

Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery T, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ (2003) A novel coronavirus associated with severe acute respiratory syndrome. New Engl J Med 348(20):1953–1966. doi:10.1056/NEJMoa030781

Kurth A, Kohl C, Brinkmann A, Eibling A, Harper JA, Wang LF, Muhldorfer K, Wibbelt G (2012) Novel paramyxoviruses in free-ranging European bats. PLoS One 7(6):e38688. doi:10.1371/journal.pone.0038688

Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, Wong SS, Leung SY, Chan KH, Yuen KY (2005) Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci USA 102(39):14040–14045. doi:10.1073/pnas.0506735102

Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Delicat A, Pawska JT, Gonzalez JP, Swanepoel R (2005) Fruit bats as reservoirs of Ebola virus. Nature 438(7068):575–576. doi:10.1038/438575a

Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, Formenty P (2009) Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis 9(6):723–728. doi:10.1089/vbz.2008.0167

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310(5748):676–679. doi:10.1126/science.1118391

Mayer F, Brunner A (2007) Non-neutral evolution of the major histocompatibility complex class II gene DRB1 in the sac-winged bat Saccopteryx bilineata. Heredity (Edinb) 99(3):257–264. doi:10.1038/sj.hdy.6800989

Muhldorfer K, Wibbelt G, Haensel J, Riehm J, Speck S (2010) Yersinia species isolated from bats, Germany. Emerg Infect Dis 16(3):578–580. doi:10.3201/eid1603.091035

Muhldorfer K, Schwarz S, Fickel J, Wibbelt G, Speck S (2011a) Genetic diversity of Pasteurella species isolated from European vespertilionid bats. Vet Microbiol 149(1–2):163–171. doi:10.1016/j.vetmic.2010.10.002

Muhldorfer K, Speck S, Kurth A, Lesnik R, Freuling C, Muller 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. doi:10.1371/journal.pone.0029773

Muhldorfer K, Speck S, Wibbelt G (2011c) Diseases in free-ranging bats from Germany. BMC Vet Res 7:61. doi:10.1186/1746-6148-7-61

Muller MA, Paweska JT, Leman PA, Drosten C, Grywka K, Kemp A, Braack L, Sonnenberg K, Nedrig M, Swanepoel R (2007) Coronavirus antibodies in African bat species. Emerg Infect Dis 13(9):1367–1370. doi:10.3201/eid1309.070342

Negredo A, Palacios G, Vazquez-Moron S, Gonzalez F, Dopazo H, Molero F, Jutste J, Quetglas J, Savji N, de la Cruz MM, Herrera JE, Pizarro M, Hutchison SK, Echevarria JE, Lipkin WI, Tenorio A (2011) Discovery of an ebolavirus-like filovirus in Europe. PLoS Pathog 7(10):e1002304. doi:10.1371/journal.ppat.1002304

Omatsu T, Bak EJ, Ishii Y, Kiyuwa S, Tohyo Y, Akashi H, Yoshikawa Y (2008) Induction and sequencing of Rousett bat interferon alpha and beta genes. Vet Immunol Immunopathol 124(1–2):169–176. doi:10.1016/j.vetimm.2008.03.004

Onyango CO, Opoka ML, Ksiazek TG, Formenty P, Ahmed A, Tukey PM, Sang RC, Ofula VO, Konongoi SL, Coldren RL, Grein T, Legros D, Bell M, De Cock KM, Bellini WJ, Towner JS, Nichol ST, Rollin PE (2007) Laboratory diagnosis of Ebola hemorrhagic fever during an outbreak in Yambio, Sudan, 2004. J Infect Dis 196(Suppl 2):S193–198. doi:10.1086/520609
Papenfuss AT, Baker ML, Feng ZP, Tachedjian M, Crameri G, Cowled C, Ng J, Janardhana V, Field HE, Wang LF (2012) The immune gene repertoire of an important viral reservoir, the Australian black flying fox. BMC Genomics 13:261. doi:10.1186/1471-2164-13-261

Paul BN, Chakravarty AK (1987) Phytohaemagglutinin mediated activation of bat (Pteropus giganteus) lymphocytes. Indian J Exp Biol 25(1):1–4

Pfefferle S, Oppong S, Drexl er JF, Gloza-Rausch F, I spen A, S eebens A, Muller MA, Annan A, Vallo P, Adu-Sarkodie Y, Kruppa TF, Drosten C (2009) Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. Emerg Infect Dis 15(9):1377–1384. doi:10.3201/eid1509.090224

Pulliam JR, Epstein JH, Dushoff J, Rahman SA, Bunning M, Jamaluddin AA, Hyatt AD, Field HE, Dobson P, Daszak P (2012) Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. J R Soc Interface 9(66):89–101. doi:10.1098/rsif.2011.0223

Reusken CB, Lina PH, Pielaat A, de Vries A, Dam-Deisz C, Adema J, Drexl er JF, Drosten C, Kooi EA (2010) Circulation of group 2 coronaviruses in a bat species common to urban areas in Western Europe. Vector Borne Zoonotic Dis 10(8):785–791. doi:10.1089/vbz.2009.0173

Rihtaric D, Hostnik P, Steyer A, Grom J, Toplak I (2010) Identification of SARS-like coronaviruses in horseshoe bats (Rhinolophus hipposideros) in Slovenia. Arch Virol 155(4):507–514. doi:10.1007/s00705-010-0612-5

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. J Virol 76(22):11476–11483

Rodriguez JJ, Dechmann DK, Voigt CC, Sommer S (2011) Identification of the nuclear export signal and STAT-binding domains of the Nipah virus V protein reveals mechanisms underlying interferon evasion. J Virol 78(10):5358–5367

Sarkar SK, Chakravarty AK (1991) Analysis of immunocompetent cells in the bat, Pteropus giganteus: isolation and scanning electron microscopic characterization. Dev Comp Immunol 15(4):423–430

Sasaki M, Setiyono A, Handharyani E, Rahmadani I, Taha S, Adiani S, Subangk it M, Sawa H, Nakamura I, Kimura T (2012) Molecular detection of a novel paramyxovirus in fruit bats from Indonesia. Virol J 9:240. doi:10.1186/1743-422X-9-240

Schad J, Dechmann DK, Voigt CC, Sommer S (2011) MHC class II DRB diversity, selection pattern and population structure in a neotropical bat species, Noctilio albiventris. Heredity (Edinb) 107(2):115–126. doi:10.1038/hdy.2010.173

Schountz T, Calisher CH, Richens TR, Rich AA, Doty JB, Hughes MT, Beaty BJ (2007) Rapid field immunoassay for detecting antibody to Sin Nombre virus in deer mice. Emerg Infect Dis 13(10):1604–1607. doi:10.3201/eid1310.070383

Shaw TL, Srivastava A, Chou WC, Liu L, Hawkinson A, Glenn TC, Adams R, Schountz T (2012) Transcription sequence of sequencing and annotation for the jamaican fruit bat (Artibeus jamaicensis). PLoS One 7(11):e48472. doi:10.1371/journal.pone.0048472

Shirato K, Maeda K, Tsuda S, Suzuki K, Watanabe S, Shimoda H, Ueda N, Iha K, Taniguchi S, Kyuwa S, Endoh D, Matsuyama S, Kurane I, Saito M, Morikawa Y, Akashi H, Mizutani T (2012) Detection of bat coronaviruses from Miniopterus fuliginosus in Japan. Virus Genes 44(1):40–44. doi:10.1007/s11262-011-0661-1

Sohayati AR, Hassan L, Sharifah SH, Lazarus K, Zaini CM, Epstein JH, Shamsyul Naim N, Field HE, Arshad SS, Abdul Aziz J, Daszak P (2011) Evidence for Nipah virus recrudescence and serological patterns of captive Pteropus vampyrus. Epidemiol Infect 139(10):1570–1579. doi:10.1017/S0950268811000550

Stockman LJ, Haynes LM, Miao C, Harcourt JL, Rupprecht CE, Ksiazek TG, Hyde TB, Fry AM, Anderson LJ (2008) Coronavirus antibodies in bat biologists. Emerg Infect Dis 14(6):999–1000. doi:10.3201/eid1406.070964

Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PB, 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 Pathog 5(7):e1000536. doi: 10.1371/journal.ppat.1000536

van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbunova MA, Snijder EJ, Fouchier RA (2012) Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. MBio 3(6):e00473–12. http://www.ncbi.nlm.nih.gov/pubmed/23170002. doi: 10.1128/mBio.00473-12

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. doi: 10.1007/s11262-009-0368-8

Wibbelt G, Kurth A, Yasmum N, Bannert M, Nagel S, Nitsche A, Ehlers B (2007) Discovery of herpesviruses in bats. J Gen Virol 88(Pt 10):2651–2655. doi: 10.1099/vir.0.83045-0

Wilkinson DA, Temmam S, Lebarbenchon C, Lagadec E, Chotte J, Guillebaud J, Ramsindrazana B, Heraud JM, de Lamballerie X, Goodman SM, Dellagi K, Pascalis H (2012) Identification of novel paramyxoviruses in insectivoros bats of the Southwest Indian Ocean. Virus Res 170(1–2):159–163. doi: 10.1016/j.virusres.2012.08.022

Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, Bai R, Teng JL, Tsang CC, Wang M, Zheng BJ, Chan KH, Yuen KY (2012) Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol 86(7):3995–4008. doi: 10.1128/JVI.06540-11

Zhou P, Cowled C, Marsh GA, Shi Z, Wang LF, Baker ML (2011) Type III IFN receptor expression and functional characterisation in the pteropid bat, Pteropus alecto. PLoS One 6(9):e25385. doi: 10.1371/journal.pone.0025385