Review

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Bats as 'special' reservoirs for emerging zoonotic pathogens

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The ongoing West African Ebola epidemic highlights a recurring trend in the zoonotic emergence of virulent pathogens likely to come from bat reservoirs that has caused epidemiologists to ask 'Are bats special reservoirs for emerging zoonotic pathogens?' We collate evidence from the past decade to delineate mitochondrial mechanisms of bat physiology that have evolved to mitigate oxidative stress incurred during metabolically costly activities such as flight. We further describe how such mechanisms might have generated pleiotropic effects responsible for tumor mitigation and pathogen control in bat hosts. These synergisms may enable 'special' tolerance of intracellular pathogens in bat hosts; paradoxically, this may leave them more susceptible to immunopathological morbidity when attempting to clear extracellular infections such as 'white-nose syndrome' (WNS).

An ancient history with a new relevance

The association between bats and human disease has been acknowledged for over a century, since the first identification of rabies *Lyssavirus* in asymptomatic vampire bats in 1911 [1]. Until recently, rabies dominated the scientific literature on bats and disease; however, following the emergence of horse- and human-infecting Hendra virus from Australian flying foxes in 1994 [2], bats have emerged as potential reservoirs (see Glossary) for a broad variety of zoonotic infections involving particularly virulent – and often fatal – RNA viruses. Although isolation of live virus from bat hosts has proven elusive in certain cases – notably that of Ebola [3] – major evidence supports the role of bats as reservoirs for Hendra and Nipah henipaviruses, Ebola and Marburg filoviruses, and severe acute respiratory syndrome (SARS) and likely Middle East respiratory syndrome (MERS) coronaviruses (CoVs), as well [4,5]. Following the identification of *Rhinolophus* spp. bats as reservoirs for SARS-CoV [6], Calisher *et al.* explored links between bats and emerging viruses in a review highlighting various aspects of bat life history and ecology – including ability to fly, dependency on torpor and/or hibernation, long life span, and gregarious social structure – that are likely to influence bats' roles as viral reservoirs [4]. In an attempt to explain bats' tolerance of otherwise virulent viruses, these earlier authors emphasized a deep coevolutionary relationship between bat genomes and those of lyssaviruses [7] and henipaviruses [8]. We explore evolutionary mechanisms enabling this immunological tolerance in bats that may be lacking in non-volant mammals, including humans.

Bats as special reservoirs for viral pathogens

Recent studies have confirmed an ancient phylogenetic relationship between bats and a suite of other viral pathogens: in addition to lyssaviruses and henipaviruses, bats are now posited as the most ancestral host taxon for the entire family of paramyxoviruses (of which henipaviruses represent one genus [9]), as well as for CoVs [10], hepadnaviruses related to human hepatitis B virus [11], and hepaciviruses related to hepatitis C virus [12]. Bats also demonstrate deep phylogenetic relationships with influenza A virus [13], filoviruses [14], and simplex viruses [15]. Several authors have compiled informative reviews [16–18] and meta-analyses [19,20] from various perspectives, continually asking 'Are bats special in their reservoir roles for zoonotic pathogens?' Common threads prevail and, increasingly, the consensus seems to be a complex and qualified 'yes'.

Although not the most represented mammalian order among zoonotic hosts, bats host more zoonotic viruses per species than do rodents and most of the resulting zoonoses have been high-profile spillover incidents of extreme human pathogenicity [21]. Bats largely host viral pathogens without demonstrating ostensible disease [22], but the pathogenicity is complex and research into impacts of viral infection on bat fitness, particularly with respect to fecundity or longevity, has been critically lacking. Obvious exceptions to viral asymptomaticity in bats include, notably, rabies and Tacaribe virus, a South American *Arenavirus* that caused widespread bat mortality in the 1950s and in later experimental infections [23]. Adenoviruses have also been linked to bat mortality [24], although such patterns are perhaps unsurprising given the disparity between this gastrointestinal system-infecting DNA virus and other bat-affiliated (mostly RNA) viruses discussed here. One study demonstrated seasonal amplification of RNA viruses, but not DNA viruses, in a monitored insectivorous bat colony in Europe [25], suggesting that different mechanisms of control may be at play for RNA versus DNA viruses in bat hosts. Additionally, a novel filovirus, Lloviu virus, was recently detected in bat tissues following

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Glossary

Adaptive immune system: one of the two main immunity strategies of vertebrates. Relies on acquired immunological memory (circulating antibodies and T and B memory cells) left over from previous encounters with a specific pathogen to promote B cell proliferation and appropriate T cell differentiation to clear a new infection. Comprises both humoral and cell-mediated components.
Antibodies: also known as immunoglobulins, these Y-shaped glycoproteins are produced by plasma cells (the daughter cells of B cells) in the humoral immune system in response to an encounter with a specific pathogen. Antibodies are found both free floating and attached to B cells and are retained in the humor after a pathogen is cleared in case of future infections.
Apoptosis: a process of programmed cell death in which the mitochondria of stressed or damaged cells initiate a signaling cascade that induces the damaged cell to burst. Resulting apoptotic bodies are then cleared by phagocytes with no harm to the host. NO and other forms of mitochondrial ROS are heavily implicated in the initiation of pathways to apoptosis.
Autophagy: a catabolic process by which lysosomes recycle internal cellular components that are unnecessary or damaged. When cell damage exceeds the ameliorating capabilities of autophagy, pathways to apoptosis are induced.
Cell-mediated immunity: the components of the immune response performed by immune cells rather than antibodies. In the innate immune system, this refers primarily to phagocytes such as neutrophils and monocytes and to cytotoxic NK cells. In the adaptive immune system, this involves cytotoxic T lymphocytes (also known as $CD8^+$ T cells), which are specific to a remembered pathogen.
Cytokines: a class of small proteins involved in cellular signaling. Cytokines are particularly important in the immune system and signal for the proliferation or recruitment of various immune cells to an infection site. They are produced by a broad range of immune cells that they signal, including macrophages, B lymphocytes, and T lymphocytes.
Humoral immunity: the aspects of immunity mediated by macromolecules rather than cells, found in extracellular fluid. In the innate immune system, this refers to the body's complement system of antimicrobial peptides; in the adaptive immune system, it primarily describes the functions of secreted antibodies.
Immunological tolerance: the failure of the immune system to mount an immune response against a recognized antigen. There is both 'self' tolerance, in which the immune system fails to attack its own proteins, and induced tolerance resulting from previous exposure to an exogenous antigen. Hosts and pathogens with deep coevolutionary relationships may share elements of their genomes, sometimes allowing the host to tolerate the pathogen as self.
Immunopathology: the detrimental effects that the host immune system inflicts on the host itself as a result of overzealous attempts to clear infection with a given pathogen. Immunopathology typically involves extensive inflammation as immune cells are over-recruited to an infection site.
Innate immune system: the second of the two main immunity strategies of vertebrates. The innate immune system is the body's first line of nonspecific defense against pathogen attack and comprises both humoral components (the complement cascade) and cell-mediated components (recruitment of nonspecific phagocytes and NK cells to infection sites).
Lymphocyte: refers to any of three types of white blood cell localized in the lymphatic fluid of the vertebrate immune system: (i) NK cells of the innate immune system; and (ii) T cells and (iii) B cells of the adaptive immune system. They are distinct from other white blood cells (chiefly macrophages) that are localized in the blood.
Mitophagy: the process by which autophagic processes are targeted to mitochondria.
Nitric oxide synthase (NOS): an enzyme that catalyzes the production of NO from the amino acid L-arginine. NO is an important cellular signaling molecule as well as a form of endogenous ROS. NOS is produced by host immune cells recruited during induction of the cytokine IFN-γ.
Reactive oxygen species (ROS): chemically reactive molecules containing oxygen that are important in intracellular signaling yet also cause damage to cell structures and both mitochondrial and nuclear DNA. Endogenous forms of ROS are produced by the mitochondria as a byproduct of metabolism and exogenous forms accrue in the cell from contact with pollutants or radiation.
Reservoir host: a species that serves primarily as a maintenance host for a pathogen. The species typically experiences minimal-to-no morbidity or mortality as a result of infection.

a massive die-off event [26], and although originally cited as the cause of bat mortality Lloviu virus has now been resolved to be a uniquely bat-adapted virus, leading researchers to explore other mechanisms for bat mortality in this incident [27]. In one study examining causes of mortality in 486 deceased bats in Europe, viral infections (lyssaviruses and adenoviruses) were responsible for only five of 144 identified disease-related deaths [28].

Bats as not-so-special reservoirs for non-viral pathogens

Recent work has begun to investigate the role of bats as hosts for non-viral pathogens, with somewhat varied results. Similar to viruses, bats demonstrate coevolutionary associations with intracellular malarial protozoa [29] as well as extracellular trypanosome protozoa including *Trypanosoma cruzi*, the causative agent in zoonotic Chagas disease [30]. Bats also exhibit coevolutionary specificity with erythrocytic *Bartonella* spp. bacteria [31]. On a par with viruses, bats appear to host both classes of protozoa and *Bartonella* spp. without ostensible disease symptoms, yet they exhibit pronounced pathology following infection with certain extracellular pathogens, chiefly *Borellia* spp. [32] and some enteric bacteria [33]. Bats also experience pathology on infection with the bacterium *Pasteurella multocida* [34], which can function as both an intra- and extracellular pathogen. Table 1 summarizes bat-hosted pathogens by clade and offers examples to illustrate what is currently known of their affiliated pathogenicity.

In addition to supporting microparasitic viruses, protozoa, and bacteria, bats are hosts for various macroparasitic helminths, chiefly trematodes [35], nematodes (including some filarial species [36]), and cestodes [37]. Bat susceptibility to helminths appears consistent with that of other mammals, which exhibit dose-dependent morbidity rather than mortality. Curiously, some hibernating bats display idiosyncratic patterns of helminth retention that differ from typical patterns of voidance during hibernation in other mammals [38].

Bats have also been long recognized as sources of the globally distributed zoonotic fungus *Histoplasma capsulatum*, which, as an intracellular parasite of macrophages [39], is asymptomatic in the chiropteran host. By contrast, when experimentally introduced via intraperitoneal inoculation, the pathogen overwhelms the extracellular spaces of bat tissues, causing lesions and severe inflammation [40]. This pronounced immunopathological response to fungal infection is particularly germane to the current widespread infection of North American bats with the extracellular fungus *Pseudogymnoascus destructans* (the causative agent of WNS). Histological wing lesions characteristic of WNS suggest massive immunopathological inflammation when hibernating bats infected with *P. destructans* arouse from torpor [41].

Thus, the ubiquitous role of bats as special pathogen reservoirs is called into question when pathogens beyond the 'virosphere' are considered; bats exhibit standard-to-extreme pathology following infection with certain bacteria and fungi. In particular, this comparison highlights the unique resilience of bats to infection with intracellular pathogens – a category encompassing all viruses, some protozoa, and some bacteria. By contrast, pathogens that predominantly occupy the extracellular space present considerable challenges for bat immune systems. In the case of trypanosomes, it should be noted that, although largely extracellular, trypanosomes also support an intracellular, amastigote life stage that is subject to the majority of immunological attack and regulation [42]. In the following sections, we explore unique mechanisms linking immune functioning with bat metabolism and longevity to offer a