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The spread and evolution of rabies virus: conquering new frontiers

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Abstract

Rabies, the most lethal zoonotic disease, is caused by lyssaviruses, most often by rabies virus (RABV). Despite control efforts, sporadic outbreaks in wildlife populations are largely unpredictable, underscoring our incomplete knowledge of what governs viral transmission and spread in reservoir hosts. Furthermore, the evolutionary history of RABV and related lyssaviruses remains largely unclear. Robust surveillance efforts combined with diagnostics and disease modeling are now providing insights into the epidemiology and evolution of rabies. The contributions of host [G] immune status, nature of exposure and strain [G] differences all clearly influence infection and transmission dynamics. In this Review, we focus on wildlife rabies, and synthesize current knowledge in the rapidly-advancing fields of rabies epidemiology and evolution, and advocate for multi-disciplinary approaches to advance our understanding of this disease.

Abstract

Much of the molecular virology of rabies virus has been well-characterized, and we are now gaining a better understanding of how nuanced infection dynamics and immune status relates to transmission. In this Review, Schnell and colleagues review our current knowledge of rabies virus transmission, spread and evolution, and they explore their determining factors.

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Author contributions

C.R.F. and D.G.S. researched data for the article. C.R.F., D.G.S. and M.J.S. made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.

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Introduction

Rabies disease, a lethal encephalitis, of which the causative agent is believed to be rabies virus (RABV), has been documented in numerous historical records dating back at least 4000 years and spanning continents[1–4]. Despite substantial advances in understanding RABV biology, a vaccine regimen that can protect against disease both before and after exposure, and mass vaccination campaigns designed to diminish the circulation of RABV in wild and domestic carnivoran reservoirs [G] the virus continues to establish itself in new host species and geographical areas, threatening animal and human lives.

In the twentieth-century, efforts to defeat rabies were ostensibly successful: North America saw a drop in annual human deaths to single-digit numbers by the 1990s[5], and Western Europe, where RABV infections in foxes was once rampant, is now considered ‘rabies free’[6]. However, in North America, epizootics in raccoons, skunks, foxes and bats belie progress made[7–11]. Moreover, RABV vampire bat variants [G] have spread in South America, new RABV lineages have been discovered, expanding the list of reservoir hosts, and efforts to eliminate RABV infections in domestic dogs have failed, posing a sustained threat of re-emergence[10,12,13]. In Europe, Africa, Asia and Australia, RABV-related lyssaviruses, which cause the same fatal disease as RABV, circulate in bats, and RABV-targeted control efforts do not always protect against these viruses[11,14]. Across the globe, particularly in Africa, Asia and India, domestic dog-adapted RABV wages a neglected epidemic that claims an estimated 59,000 human lives annually[15,16].

How RABV establishes transmission cycles in new hosts is mostly unknown. Moreover, how such host shifts can be prevented remains an open question. There are dozens of RABV strains, each of which associate closely with a host mammal species in either the chiroptera or carnivora orders. Mechanisms of transmission and evolution may differ within each virus-host relationship. More importantly, it is unclear what prevents RABV from adapting to species of other mammalian orders. Although cross-species transmissions usually result in ‘dead-end’ infections, exceptional host shifts continue to occur in unpredicted ways. Although our understanding of RABV-related lyssaviruses continues to grow (Box 1)[17–21], how lyssaviruses circulate and their potential for outbreak remain to be fully established, as their pathology is indistinguishable from that of RABV[22]. It is almost certain that infection with non-RABV lyssaviruses occurs but goes unnoticed based on the lack of discriminatory diagnostic tools in many countries[23]. This assumption is supported by the finding that even infection with RABV has been misdiagnosed as malaria encephalitis in children[24]. Importantly, some lyssaviruses are divergent enough to resist protection from current vaccines and rabies immunoglobulin (RIG)[25–27].

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The current distribution and impact of RABV across the globe contrasts with how it has historically been studied: foundational understanding of RABV spread and transmission comes from studies on RABV infections in wildlife in North America and Western Europe, but RABV infections in canines in Africa and Asia, which is responsible for the vast majority of human fatalities, has been less studied. Similarly, our understanding of RABV biology largely stems from mouse models with a relatively small repertoire of strains. In both investigating the evolutionary history of lyssaviruses and working towards its elimination, our understanding benefits from a convergence of virology, epidemiology and ecology. In this Review, we first briefly discuss RABV biology for context, highlighting relevant new research. We then discuss RABV transmission, and explore enzootic maintenance and epizootic spread of the virus. Finally, we review molecular evolutionary dynamics, host adaptation and the origins of RABV.

Rabies virus biology

Molecular virology and life cycle.

RABV is a negative-stranded RNA virus of the *Rhabdoviridae* family[28,29]. RABV virions [G] are enveloped by a host cell-derived membrane and take on a bullet shape of about 200 nm by 80 nm. The bullet shape is likely influenced by the constraints of budding[30] and viral uptake[31]. The relatively small RNA genome of the virus (~12 kb) encodes for five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and polymerase (L, in reference to the large size of the gene)[28] (Figure 1A). The basic functions of lyssavirus proteins are well-conserved (Box 2).

The rabies life cycle and its resulting pathogenesis have been extensively studied and reviewed[32–35]. Briefly, RABV, with its modest genome and single surface glycoprotein, can infiltrate an astonishing number of mostly neuronal tissues in almost any mammal to induce its lethal pathology. This might lead one to assume that the receptor that RABV uses to enter the cells is exceptionally conserved. However, debate surrounds RABV receptor binding (Fig. 1b). *In vitro* and knockout *in vivo* experiments have homed in on three primary candidates: the nicotinic acetylcholine receptor (nAChR), neural cell adhesion molecule (NCAM, also known as CD56), and the low-affinity neurotrophin receptor, $p75^{NTR}$ [36]. Overall consensus holds that glycoprotein can bind flexibly to all three receptors, and possibly others, as needed in different stages of the life cycle or in different hosts. Recent studies on virus internalization confirm that RABV uptake is mediated by classic clathrin-mediated endocytosis (Fig. 1B)[37,38].

Infection usually begins in muscle tissue following a bite from an infected animal. The virus then crosses neuromuscular junctions to peripheral nerves and uses retrograde axonal transport to reach the central nervous system (CNS)[39]. It was originally believed that RABV did not replicate in muscle, but evidence now shows that replication occurs in some instances in muscle[40]. It is still unclear why RABV is not detected immediately after exposure, especially if it is replicating in muscle tissue; strong immune evasion is likely to be responsible (see below). Evidence suggests that the virus takes multiple routes to reach the CNS (such as via motor or sensory neurons), depending on the site of inoculation and post-infection time[41].

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Whereas the initial spread of RABV is exclusively retrograde, at the end stage of infection, RABV reverses its direction of transport and migrates out of the CNS in an anterograde fashion towards the periphery[42]. Centrifugal spread, especially to the salivary glands where the virus can easily transmit through a bite, is essential to restart the cycle. For much of its life cycle, RABV infection is asymptomatic, and clinical signs of neuronal dysfunction present only at late stages of the disease. Rabies disease then rapidly deteriorates the health of the host and leads to death from respiratory or heart failure, usually within days[35]. A few unusual examples of survival have been reported in humans, but interventions have not proven repeatable[43,44].

Neurological symptoms can manifest in different ways. Often, infected animals (including humans) exhibit the 'furious' form of rabies disease, which is characterized by agitation and aggression[45]. In other cases, the disease manifests as paralysis (paralytic rabies), and sometimes the two forms alternate during the course of disease[45]. Variations in immune responses may have a role in the manifestation of disease[46]. Recent studies using RABV isolates [G] from canines exhibiting furious and paralytic disease strengthen anecdotal evidence that strains may also contribute to these phenotypes[47]. A distinctive hallmark of rabies disease is hydrophobia, in which the patient is extremely resistant to consuming, or even encountering water or other liquids. The neuropathogenesis, diagnosis and management of rabies disease in humans has been reviewed previously[48].

It is becoming increasingly clear that the pace of rabies infection is determined in part by viral factors that differ between strains[49], and possibly between lyssavirus species. Recent work comparing bat- and carnivore-derived variants in mice found increased neuroinvasiveness and lethality of the bat variant[50], a pattern which may ultimately influence the clinical outcome and diagnosis of rabies[51]. However, though well-established, the use of mouse models to test the pathogenicity and neuroinvasiveness of lyssaviruses from diverse reservoirs tells an incomplete story. Experimental infections in bats suggest that, upon RABV exposure, they develop strong but sometimes sub-detectable immunological memory[52–54]. Such experiments highlight both the utility of examining variant phenotype in a host closer to its natural reservoir, as well as suspected differences between bat and terrestrial mammal immunity(Figure 2)[55]. The dose of RABV administered during an exposure is also important[52]. Higher doses result in shorter incubation times (the time between exposure and the infectious period) and, consequently, more rapid deterioration of the host. Lower doses seem to lengthen the incubation time and possibly increase chances of an abortive infection, or an infection in which the virus enters the host but cannot replicate sufficiently to invade the CNS. Of note, the inoculation titers are likely to be lower in bats than in larger animals[51,52,56].

Immune responses to RABV and immune evasion.

The skin is the first defense barrier against RABV. Except for relatively rare instances of intranasal inoculation[57], a breach in the skin is essential for RABV to penetrate the host. After penetration, RABV infections elicit inadequate immune responses until late stages of the disease. Indeed, a major reason for the lack of symptoms during the incubation period is

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the absent immune response[58]. RABV adeptly evades innate immunity both actively at the cellular level[59,60], and via its tropism to the immunologically-privileged nervous system.

Investigating direct and indirect immune evasion mechanisms of RABV has been a particularly active area of research in recent years (recently reviewed in REF.[61]). Each of the five proteins encoded in the RABV genome has been implicated in immune suppression either directly or indirectly, although phosphoprotein has a key role and can differ in its activity between strains[62]. Immune dysfunctions linked to RABV include inhibition of interferon (IFN) signaling, systemic immunosuppression, altered nitric oxide production and mitochondrial dysfunction[61,63]. RABV has also been shown to induce apoptosis, the benefit or detriment of which to the virus is currently debated[61]. As a consequence of the innate immune evasion mechanisms, the host does not mount an adaptive immune response against the virus until late stages of the disease, when the viral load is intractably high[35]. The lack of adaptive immunity until it this late stage of infection is particularly unfortunate as RABV is highly immunogenic when administered in a vaccine format and, furthermore, protective immunity is relatively straightforward.

Upon vaccination, T cell-dependent humoral immunity generates virus-neutralizing antibodies (VNAs) that are the critical correlate of protection, as determined by extensive experimental infection studies and clinical observations[58,63]. Although RABV can induce cytotoxic T cells, these cells do not to have a substantial role in protection from rabies disease[58]. Antibodies can be found against multiple RABV proteins, but only VNAs against glycoprotein are relevant for protection. VNA-mediated clearance is believed to function by coating the virion and blocking receptor binding, however cell-mediated antibody functions have never been definitely ruled out[64]. Understanding of RABV neutralization is complicated by the fact that infection occurs primarily in the nervous system, and uncertainty persists surrounding viral clearance from the CNS[65]. Those mechanisms will need elucidation before a therapeutic can be developed that works after the onset of symptoms (reviewed in REFS.[58,63]). Notably, similar to the study of lyssavirus strain pathogenicity, our understanding of lyssavirus-induced immunity mostly comes from mouse studies, which do not necessarily recapitulate natural infection. Nevertheless, well-established methods of assaying for VNAs[66] informed the highly successful post-exposure prophylaxis (PEP) that has been widely used for decades.

There is a tendency to regard RABV infection in a binary fashion: virus neutralization by antibodies if they are present, and lethal infection if they are not. Infection dynamics are likely more nuanced, as a combination of low exposure dosages, functional innate immunity, and neutralizing antibodies can result in abortive infection. A high frequency of abortive infection is clearly seen in the presence of antibody in healthy bats[67–69], spanning many species and geographic areas[52]. VNAs in non-bats are less well-established, but have been detected at varying levels in diverse reservoir and non-reservoir species, including human, cow, and mongoose[45,70–72]. These findings highlight major unanswered questions: what are the biological factors underpinning whether hosts survive or succumb to rabies? and if naturally acquired VNAs are protective, what role do they have in the long-term perpetuation of RABV? It remains unclear why lethality, which seems unlikely to benefit the virus, is highly permissible in its transmission dynamics.

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Transmission, maintenance and spread

RABV transmission.

Transmission of RABV, and indeed all lyssaviruses, occurs through contact between infectious saliva and broken skin or mucous membranes, typically via bites, but also through scratches or licks. Transmission through consumption of carcasses or infected animals may also occur, but neutralization of RABV in the gastro-intestinal tract means that abrasions in the oral cavity are likely to be required to give the virus passage into innervated muscle or neuronal tissue[73,74]. Experimental infection directly into the intestine resulted in neither clinical rabies disease nor development of VNAs[75]. Examples of aerosol transmission have been limited to extraordinary situations involving high concentrations of virus, such as in the largest aggregations of free-tailed bats (numbering in the millions) or in laboratory settings[57,76,77], which suggests this route is unlikely to be important for natural virus circulation. In regions where RABV infection in human is rare, there have been instances of misdiagnosis and subsequent RABV transmission via organ transplantation upon death of the patient[78,79].

The host immune status and the nature of the exposure (for example, dose, location, and depth of inoculation) can influence whether an exposure results in a lethal or abortive infection. Strain-level differences among RABVs that influence the outcome of exposures are increasingly evident, particularly in comparisons between bat and terrestrial carnivore-associated variants[51]. For example, silver-haired bat rabies virus (SHBRV), but not dog-derived RABV isolates, are capable of hematogenous spread in mice after intravenous inoculations, which suggests that viremia could be an alternate route to the CNS in some strains[80]. SHBRV also replicates better at lower temperatures in epithelial cells than carnivore RABVs, which may indicate the adaptation to the relatively shallow bites of small insectivorous bats[81]. In mice, bat-derived RABVs generally seem to be less neurotropic and less uniformly lethal than carnivore RABVs, which may explain why the rare instances of human survival of clinical rabies mostly involved bat variants[82]. Mechanistic explanations for differences in pathogenesis and virulence at the cellular and molecular level remain largely elusive, limiting our ability to understand the risks posed by newly discovered or emergent strains. Combining new genomics technologies with classic phenotypic studies may enable researchers to identify how viral genomic backgrounds interact with host transcriptomic responses to infection, ultimately leading to the identification of factors that influence the outcomes of rabies infections.

Enzootic maintenance of RABV.

RABV is an obligately lethal pathogen that infects relatively long-lived, slow-reproducing mammalian hosts, has an infectious period spanning less than 1 week (and typically only 2–4 days), and relies on transmission among members of the same specifics to be maintained at the population level. In theory, this combination of characteristics would be expected to rapidly deplete susceptible individuals and cause host and/or virus extinction[83]. Indeed, estimates of the basic reproductive number (R_0, the number of secondary infections generated from a single infection in an entirely susceptible population) of RABV are near or below the theoretical limit precluding epidemic spread ($R_0 = 1$) in domestic dogs, wild

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carnivores and bats, suggesting that RABV should be prone to stochastic extinctions and sensitive to control measures[7,84,85]. Explaining how RABV is perpetuated over long time periods has therefore been a major conundrum that directly affects policies for rabies control through vaccination and culling of reservoir hosts.

Fortunately, the remarkable volume and quality of data collected on RABV through public health and veterinary surveillance systems has made the virus a model system for understanding the epidemiological dynamics of zoonoses within their animal reservoirs (Box 3). On the basis of multi-annual epidemic cycles, early studies suggested that the maintenance of RABV was driven by an interaction between density-dependent transmission and rabies-induced mortality, whereby reduction of host populations owing to lethal infection dampened transmission and enabled the recovery of susceptible hosts[86–88]. However, the consistent failure of population reduction (that is, culling) to control RABV transmission and weak or absent empirical relationships between population density and measures of RABV transmission[84,89,90] raised doubts that a simple relationship between density and transmission exists. Moreover, a recent synthesis highlighted alternative mechanisms, including demographic structure and spatial structure, that could generate observed epidemic cycles in the absence of density dependence[91]. Individual-level variation in host dispersal or propensity to bite during infection is poorly understood but could also have a crucial role in the maintenance of RABV by creating ‘super-spreaders’ of infection[84,92].

It is now evident that RABV has evolved both general and reservoir host-specific maintenance mechanisms to avoid extinction. Across RABV reservoirs, transmission is aided by the aggressive behavior of rabid animals. Disease-induced aggression may facilitate transmission at low host population densities and for reservoirs that have infrequent natural contacts with conspecifics (for example, solitary bat species) or territorial carnivores that live in small groups (for example, arctic foxes). Variable incubation periods are another general feature that may help RABV avoid extinction. Although incubation periods are typically 1–3 months, deaths in wild-caught, captive bats show longer delays (>200 days) are likely to occur in nature[52,93,94], which might promote viral dispersal to new host populations or the recovery of susceptible hosts through births or immigration[93,95]. Finally, across many reservoirs, transmission dynamics and prospects for control of host populations can be profoundly influenced by the spatial structure, such as induced by rivers, mountains and other landscape barriers, vaccination campaigns or reliance on human-provided resources[96].

Given the diverse ecologies of its bat and carnivore reservoirs, it is not surprising that RABV has evolved distinctive strategies for enzootic maintenance in different reservoirs (Fig. 3, Fig. 4). For example, in temperate, hibernating bats, RABV overwinters by slowing replication, thereby prolonging the incubation until spring birth pulses replenish susceptible individuals to the population[97–99]. By contrast, for tropical bats that are transmitted throughout the year, spatial processes such as metapopulation dynamics seem to prevent host or virus extinction[85]. Maintenance mechanisms for bat colonies comprising hundreds of thousands to millions of individuals remain unclear. These colonies sustain high seroprevalence [G], suggesting optimal conditions for spread, yet no die-offs from RABV

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have been observed[69,100]. Studies on European bat lyssavirus (EBLV) suggested extended infectious periods or carrier states might explain persistence in large colonies without overt mortality[101]; however, experimental models in both EBLV and RABV have not generated infections that produce long-term shedding without death[52,102,103], raising doubts on the existence or epidemiological relevance of a healthy carrier state. Among the wild and domestic carnivore reservoirs of RABV, variation in life history traits, population density, habitat use and degree of associations with humans also exert pressures that are important for maintaining viral transmission through extinction-recolonization dynamics or age-structured transmission[91,104]. The ability of RABV to maintain independent transmission cycles in such a diverse set of hosts is a remarkable testament to its epidemiological plasticity. Important questions for understanding the changing ecological niche of RABV include how this plasticity alters viral molecular evolution and whether strategies in some reservoirs predispose establishment in novel host species or landscapes.

Epizootic spread of rabies.

Despite the challenges of enzootic persistence, RABV has repeatedly demonstrated sudden opportunist epizootic spread when introduced to naive host populations. Although such spread occasionally follows cross-species transmission[105] (see below), the best-studied examples of landscape-level viral invasions from point source introductions (that is, 'travelling or epizootic waves') involve translocations of the virus into new populations of existing reservoirs by human movement of incubating animals or natural host dispersal[87]. Wavefront velocities have been quantified in foxes[86], skunks[8], raccoons[7] and vampire bats[10,106] and generally advance between 10 km and 40 km per year. Mathematical models and phylogeographic analyses of these invasions have revealed key facets of the transmission dynamics of RABV. For example, the spread of RABV among raccoons in eastern North America was partly contained by mountain ranges and rivers, enabling these natural landscape barriers to complement oral vaccination campaigns and limit western spread[107]. Invasions in other reservoirs, such as skunks and bats, have proceeded at more regular velocities along routes defined by host population structure[8,108]. In principle, predictable wavefronts enable forecasting of risk and vaccination of reservoirs, humans and domestic animals prior to viral invasion, but strategic planning in unaffected areas from model forecasts remains relatively uncommon. Moreover, intentional or unintentional translocation of synanthropic species (for example, dogs and raccoons) reduces predictability and can compromise natural or vaccine-generated barriers[109–112]. Fully understanding viral spread at the landscape level will require answers to fundamental questions: what ecological, climatic or anthropogenic factors trigger viral invasions to new areas? What explains different spread velocities within the same reservoir hosts in different geographic locations or time points in the invasion process[7,8,10]? Will RABV persist indefinitely or fade out from newly invaded areas? New technologies for studying animal movement, such as GPS tagging and nano-scale radio-transmitters, together with the comprehensive epidemiological and genomic datasets collected through surveillance systems provide exciting opportunities to resolve these questions and enlighten programs for rabies prevention and control.

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Evolution and host shift

In rare instances, spillover infections [G] of RABV are maintained over long time scales in new host species. These events are important for public health because they create new reservoirs for human exposures[113,114] and can affect wildlife conservation when new transmission cycles occur in threatened or endangered species[115] or increase spillover to those species. Most novel transmission cycles have been established in previously known hosts of RABV, such as bats, skunks and foxes[105,116]. However, evidence of sustained transmission in non-traditional hosts has reinforced the need to understand how RABV overcomes evolutionary barriers to establish novel reservoirs. These non-traditional hosts include marmosets[12] and kinkajous (primates)[117], greater kudu antelope (artiodactyl)[118], and coatis[119], mongooses[120,121] and ferret badgers[122] (carnivores).

The need for host adaptation in RABV.

Despite the apparent capacity of rabies to infect any mammal, numerous lines of evidence imply that RABV must adapt to establish in new host species[123]. From an epidemiological perspective, RABVs that use multiple host species for long-term maintenance are conspicuously absent. *In vivo* and *in vitro* studies suggest this absence may reflect genetic fine tuning of RABV to specific hosts. Phenotypic differences among genetic variants of RABV are commonly observed[81], and infections in heterologous hosts show altered patterns of infectivity and pathogenesis that would be expected to limit onward transmission. For example, raccoons inoculated with homologous RABV manifest acute clinical rabies after a long incubation period, but show subtle clinical signs such as lethargy followed by death or abortive infection when inoculated with dog or skunk-origin viruses[124,125]. Phylogenetic comparative studies provide another line of evidence that host barriers exist and must be overcome by viral evolution. In bats, both cross-species transmission and historical host shifts occurred more often between closely related species than between species with extensive geographic range or ecological overlap[116]. This suggests that although ecological opportunity is a prerequisite for emergence, physiological or immunological barriers that correlate with host relatedness influence the likelihood of successful host adaptation. Non-random clustering of RABV reservoirs on the carnivore phylogeny could reflect a similar predominance of host shifts among related hosts or variation in susceptibility among clades of carnivores[126,127].

Molecular evolutionary dynamics of rabies host shifts.

Given that RABV must evolve to efficiently infect and establish transmission in new host species, it is surprising that classic molecular evolutionary signatures of positive selection (dn/ds ratios >1) have been only rarely described in RABV. The overwhelming force on the RABV genome is purifying selection, and until recently positive selection was only detected in a small number of amino acid positions in the ectodomain of the glycoprotein[17,128]. Selection on the RABV genome may be difficult to detect, given that available sequences are typically limited to the genes encoding the nucleoprotein and glycoprotein and span only a small fraction of viral evolutionary history[129]. Moreover, potential host shifts may be driven to extinction by control efforts, such as vaccination, before adaptation occurs[105]. Another possible explanation is that most studies have used computational methods that assumed

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pervasive selection across all branches of viral phylogenies, an assumption that is unlikely to hold for a virus experiencing a sudden change in environment following long periods of relative evolutionary stasis. Indeed, more recent studies have shown pulsed episodes of positive selection on diverse sites in the nucleoprotein, glycoprotein and polymerase[130,131]. Repeated host shifts between the same donor and recipient species have shown that the adaptive evolution of RABV can even be repeatable. Independent host shifts from domestic dogs into ferret-badgers in Asia resulted in parallel substitutions in the genes encoding nucleoprotein and polymerase[130]. In the southwestern USA, three independent outbreaks of bat-associated RABV in skunks were associated with six parallel changes across the viral genome, but did not overlap with those observed in ferret-badgers[105]. Similarly, among 30 host shifts between American bats that represented different combinations of donor and recipient species, combinations of positively selected changes were largely unique to each host shift[131]. It therefore seems likely that the adaptive changes necessary for each host shift are driven by the interaction between the genetic background of the infecting virus and the identity of the host species involved.

The importance of viral genetic background implies that the degree of viral preadaptation to a particular host can make some host shifts more likely to occur than others. For example, bat viruses with threonine in position 242 of the glycoprotein have caused more outbreaks in carnivores than those of other genetic backgrounds[105]. Deep sequencing has revealed sub-consensus viral populations as another source of genomic diversity that can be selected during host shifts. For example, changes that became fixed following a host shift from skunks to foxes in California were present as low frequency variants for years before outbreaks in foxes began[132]. As a potentially important consequence of the increasing diversity of reservoirs, the virus may be exposed to previously unexplored areas of host genomic space, increasing the likelihood of rare, preadapted variants that facilitate further host range expansions, perhaps even to reservoirs outside of bats and carnivores. This ‘snowball effect’ hypothesis predicts an increasing frequency of future host shifts[123].

The unsolved mystery of rabies origins.

Phylogenetic studies have shown that the evolutionary history of RABV is dominated by host shifts, predominately within bats and within carnivores, but more rarely between these mammalian orders[17,130]. As the vast majority of lyssavirus diversity occurs in Old World bats (Figure 3A), RABV was commonly assumed to have evolved in Old World bats, shifted to carnivores and subsequently spread globally[133]. However, unexplained observations have plagued the bat-to-dog hypothesis and complicate our understanding of the evolutionary history of RABV. Most notably, Old World bats carry diverse RABV-related lyssaviruses but not RABV, whereas New World bats exclusively carry RABV (Figure 3A). By contrast, carnivores maintain RABV in both the New and Old Worlds (Figure 3B). The absence of RABV from its putative origin (Old World bats) raises the important question of how carnivores in the Old World acquired RABV. Conceivably, RABV evolved around the time of the origin of bats (~62 mya[134]) and moved with bats as they colonized the Americas from Africa or North Asia. However, this would require a RABV host shift from bats into ancestral or modern carnivores, followed by RABV extinction from Old World bats amid conditions that favored the persistence of RABV in New World bats. A more controversial

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alternative is that RABV evolved in carnivores following a host shift of a non-RABV lyssavirus from Old World bats, spread to the New World RABV infected dogs during European colonization, and more recently jumped from carnivores to bats in the New World. Although cross-species RABV transmission from carnivores to bats is unlikely, given that rabid carnivore bites would likely kill comparatively smaller bats before they contract rabies, it is worth noting that all bat RABV variants are monophyletic[130], so such an event would only need to occur once. Moreover, the recent discovery of a carnivore-related RABV in a bat in Sri Lanka may represent the first evidence of a carnivore-to-bat exposure that resulted in successful RABV infection, setting a precedent that a similar transmission event might have occurred in the New World[135]. Less speculative evidence for a more recent origin of RABV in the Americas comes from studies suggesting that RABV is not at equilibrium within American bat communities, as might be expected for an ancient virus. This evidence includes the fact that American bat RABVs are still diversifying their host range[136], geographically isolated populations of established reservoirs are rabies-free (suggesting arrival of rabies after host vicariance)[108], and ongoing invasion fronts into historically rabies-free areas resemble those observed for recent introductions[10]. This evidence suggests a more recent introduction to New World bats, but it is biased by the enhanced detection abilities of modern surveillance programs and by the changing environmental conditions that could alter the abundance and distribution of RABV reservoirs[137].

Historical records and phylogenetic studies provide little resolution of these evolutionary uncertainties. Ancient records in the Old World make reference only to rabid dogs, not bats[4,138]. In the New World, there is no clear evidence of indigenous bat or dog rabies[139,140], and the first confirmed reports of bat rabies appeared around the start of the sixteenth century as colonization began[1]. Rabies was not reported in North American bats until the 1950s[141]. However, the bias of record availability is worth noting; the Mayans were the only pre-Columbian Old World civilization to develop sophisticated writing, and most of their records were destroyed upon Spanish settlement[139]. Phylogenetic studies have clarified some aspects of the biogeography and evolution of lyssaviruses, but leave much uncertain for RABV in particular. At the genus level, a recent phylogeographic study rejected the expected African origin of lyssaviruses in favor of a Palearctic origin with multiple radiations creating the different phylogroups [G][142]. For RABV, most studies put the most recent common ancestor of bat RABV within the timescale of European colonization (~500y, consistent with a carnivore-to-bat host shift); however, molecular clock methods based on contemporary sequences are unlikely to be able to estimate the true timing of ancient host shifts because of strong purifying selection and the lack of sequences from extinct lineages[130,143]. It has been suggested that paleovirological studies of endogenous viral elements (EVEs) in host genomes might clarify the time scale of RABV origins[129]. Although rhabdoviral or rhabdovirus-like EVEs exist in insect and plant genomes[144], the absence of lyssavirus-related EVEs despite extensive screening of mammalian genomes, casts doubt on this approach to resolve RABV origins[145]. New virus discoveries and/or analytical methods may eventually solve the riddle of rabies origins.

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Surveillance, Diagnostics, and Vaccines

As discussed above, traits unique to RABV have long spurred far-reaching surveillance efforts yielding large, comprehensive data sets. These datasets have, in turn, enabled advances in disease modeling which may eventually enable accurate predictions about the movements of RABV in host populations. However, the current situation requires even more than just monitoring the dozens of known RABV strains. In the past decade, numerous novel RABVs were discovered in Latin America that form species-associated genetic clusters in unusual host species, including non-human primates[12,117] and coati[119]. In Taiwan, outbreaks of RABV in ferret-badgers have raised major public health concerns as a wildlife reservoir would compromise the long-term sustainability of dog vaccination for RABV elimination[113]. Whether these discoveries reflect spillovers from unknown reservoirs or emergent host shifts is unknown, as is the relative virulence of these viruses in humans or domestic animals.

Another frontier for surveillance lies in non-RABV lyssaviruses. At present, the discovery of RABV-related viruses [G] is usually attributed to either unusual exposures or fastidious surveillance efforts that endeavor to distinguish between strains (Box 1). The recent discovery of lyssaviruses in Spain[18] and Sri Lanka[19] highlights a potentially vast lyssavirus diversity that will continue to be discovered as surveillance systems and diagnostics become more accessible. Statistical approaches, such as machine learning, have been useful to identify unknown sources of disease in other systems[146,147] and could accelerate the search for novel lyssaviruses by predicting potential reservoirs from the traits of known reservoirs.

Apart from detection, the threat to animal and human health posed by non-RABV lyssaviruses remains unsettled. Lyssaviruses cause the same zoonotic disease but are incompletely protected by rabies vaccines or biologics, which were designed to target only RABV[14,22,25]. All current human and animal vaccines have been developed against RABV; there is limited, incidental cross-reactivity among viruses of phylogroup I and no cross-reactivity between phylogroups[26,27,148]. Rabies is responsible for an estimated 59,000 global human deaths annually[15], and an estimated 15 million people receive PEP annually for exposures[149]. However, the true disease burden of non-RABV lyssaviruses is unknown because clinical signs are indistinguishable from RABV infections and discriminatory diagnostics are rarely available for either human cases or animal surveillance[22]. Thus, although only 12 human deaths have been confirmed as being caused by six non-RABV lyssaviruses[22], this number is likely to be a large underestimation. A study in Ethiopia showed that 1% of animals diagnosed with rabiesdisease were actually infected with Mokola virus (MOKV)[23].

Investment in RABV vaccines has been touted as one of the lowest cost but highest impact tradeoffs among vaccine-preventable infectious diseases[150]. Yet, canine RABV still causes tens of thousands of human fatalities on multiple continents because mass vaccination of dogs is not sufficiently adopted and post-exposure prophylaxis of humans is either unavailable or inaccessible[15]. Looking forward, host shifts to new reservoirs bring new challenges. Lyssaviruses circulate in bats in Europe, Africa, Asia and Australia and have infected terrestrial mammals numerous times, raising the risk of novel non-RABV

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lyssaviruses in carnivores or other terrestrial mammals. Indeed, the absence of MOVK and IKOV from bats but presence in other species may indicate that such host shifts have already occurred[21,151]. Such shifts could have devastating effects given the absence of pre- or post-exposure vaccines that protect against these lyssaviruses, which are phylogenetically and antigenically divergent from RABV. Investment in studying lyssaviruses and developing a pan-lyssavirus vaccine is currently lacking, leaving the world unprepared in the event of an outbreak.

Given their prominence as reservoirs, bat populations are perhaps an attractive target for lyssavirus control. As noted, culling efforts in Latin America have failed to change RABV seroprevalence levels within the population and may even be counterproductive[90]. Mass bat vaccination has never been implemented, as it has been for terrestrial wildlife, but experimental vaccinations have been performed in captive bats, testing inactivated RAVB in Brazilian free-tailed bats[54], vaccinia-vectored live vaccines in vampire bats[152–154], and raccoonpox-vectored live vaccines in big brown bats[155] and Brazilian free-tailed bats[156]. These and other studies of bat responses to RABV[52,53] repeatedly find low or undetectable long-term antibody titers, as measured using conventional virus neutralization assay protection cutoffs defined for humans. Nevertheless, vaccines administered to bats through various routes, including topical, oral and intramuscular, protect against RABV challenge. Combined with our limited knowledge of bat immunity to rabies[55], these findings highlight the need for defined bat-specific correlates of protection before investing in mass bat vaccination. The feasibility of scalable and effective mass vaccination of wild bats must also be resolved. For social and gregarious bat species, vaccines could be applied topically and spread through social interactions (that is, allo-grooming) to maximize coverage, akin to strategies currently used to poison common vampire bats[157,158]. However, field studies and epidemiological models are needed to determine what vaccination coverage may be expected with this dissemination vehicle and what proportion of colonies would need to be treated to meaningfully reduce rabies circulation in bat populations.

The changing epidemiological face of rabies creates new challenges for using molecular data and models to inform decisions for prevention and control. Where domestic dog rabies is approaching elimination, rapid sequencing will be paramount to determine whether resurgent outbreaks represent chains of previously undetected local transmission or re-introductions from still enzootic areas. New technologies mean that rapid diagnostics and genome sequencing (that is, MinION portable, real-time DNA and RNA sequencer) can be carried out in the field in affected countries[159]. Appropriate investments to strengthen local analytical and laboratory capacity are therefore paramount for elimination.

Conclusions

Despite its long history, rabies remains an important but neglected infectious disease. Much of the molecular virology of RABV has been well-characterized, and we are now gaining appreciation of how nuanced infection dynamics and immune status relates to transmission. Enzootic maintenance of rabies within a population depends on transmission within precariously narrow windows, and it is now clear that the virus avoids extinction by both general and reservoir host-specific mechanisms with remarkable epidemiological plasticity.

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At the landscape level, mathematical models and phylogeographic analyses provide a sketch of epizootic rabies spread, but questions surrounding ecological, climatic, and anthropogenic factors remain. At an evolutionary level, there is evidence that strong barriers prevent RABV from establishing in new host species, and both ecological opportunities and viral adaptation are needed to overcome these barriers. Nevertheless, host shifts occur, and the study of molecular evolutionary dynamics has revealed the role of purifying selection and the importance of viral and host genetic backgrounds in such shifts. Further study of contemporary host shifts will likely shed light on rabies' unknown origin.

Examination of modern rabies has revealed subtleties in our understanding of how the virus moves within and among its animal reservoirs. The convergence of insights gained from field research with molecular virology studies will be key to fully elucidating rabies. Building upon the carnivore vaccination programs of the twentieth century, the next steps will be to devise strategies and technologies to similarly manage rabies within bat reservoirs; further integrate diagnostics that can rapidly differentiate strains into surveillance efforts; hone our predictive power to detect outbreaks; and coordinate local resources to halt the spread of this lethal zoonosis.

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Glossary:

Host
An individual or animal infected with a virus, such as a lyssavirus

Strain
A viral population maintained within a particular **reservoir**, often in a geographically defined area that can be genetically distinguished from other sympatric viral populations

Reservoirs
Animal pools that perpetuates the long-term transmission of a rabies virus **strain**. Sometimes called a reservoir host or maintenance host

Variants
Viral **strains** with small genetic differences that may or may not be detectable by antigenic characterization

Virions
Infectious particles, complete with the viral genome and viral proteins, capable of transmission to a new cell or **host**

Isolates
Viral samples that have been obtained from an infected individual or animal **host**

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Seroprevalence
proportion of animals or individuals presenting virus-specific antibodies in their serum, indicative of exposure to either the virus or vaccine

Spillover infections
Transmission events in which a lyssavirus **strain** successfully infects an animal of a non-**reservoir** species

Phylogroups
Subgeneric classification of lyssavirus **species** grouped by genetic and immunologic characteristics

Rabies virus-related lyssaviruses
Virus of the *lyssavirus* genus of RNA viruses other than the prototypical member, rabies virus.

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Box 1:

Discovery of rabies virus-related viruses

In 1956, a pathogenic virus was discovered in the brains of fruit bats (*Eidolon helvum*) in Nigeria[160]. The serological tests available at the time identified it as related to, but distinct from, RABV. A similar result was found after a South African man died from a rabies-like illness following a bat bite in 1970[161]. The discovery of Lagos bat and Duvenhage lyssaviruses were the first in a series of discoveries of rabies virus (RABV)-related viruses that now comprise the lyssavirus genus, which includes RABV. There are fourteen members of the genus (see table), and two recently discovered lyssaviruses: Lleida bat lyssavirus (LLEBV) discovered in Spain in 2011 and Gannoruwa bat lyssavirus (GBLV) discovered in Sri Lanka in 2014[18,19]. Lyssaviruses are further grouped into two phylogroups, an organization based on serum cross-reactivity against the viral proteins and thresholds in genetic sequence differences[14,17]. However, the divergence of Ikoma (IKOV) and West Caucasian bat (WCBV) lyssaviruses, which cluster with neither phylogroups, suggests that phylogroup organization may be more complex than originally proposed[21,25]. Non-RABV lyssaviruses have been traced almost exclusively to bats, with two notable exceptions: Mokola lyssavirus (MOKV) has been found across six African countries in shews[162], domestic dogs and cats[20,151], and was responsible for at least one human death[163]; Ikoma lyssavirus (IKOV) was recently discovered in Tanzania in an African civet, despite decades of local carnivore surveillance and research[21,164]. The persistent discovery of new lyssaviruses in Europe, Africa, Asia and Australia reflects the utility of continual RABV and lyssavirus surveillance and increasingly sensitive diagnostics, but also highlights potential unassessed risks. More members are likely to be discovered in coming years.

Phylogroup	Species name	Abbreviation
Phylogroup I	*Aravan lyssavirus*	ARAV
	Australian bat lyssavirus	ABLV
	Bokeloh bat lyssavirus	BBLV
	Duvenhage lyssavirus	DUVV
	European bat 1 lyssavirus	EBLV-1
	European bat 2 lyssavirus	EBLV-2
	Irkut lyssavirus	IRKV
	Khujand lyssavirus	KHUV
	Rabies lyssavirus	RABV
Phylogroup II	*Lagos bat lyssavirus*	LBV
	Mokola lyssavirus	MOKV
	Shimoni bat lyssavirus	SHIBV
Unclassified	*Ikoma lyssavirus*	IKOV
	West Caucasian bat lyssavirus	WCBV
	Lleida bat lyssavirus	LLEBV

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Phylogroup	Species name	Abbreviation
	Gannoruwa bat lyssavirus	GBLV

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Box 2:

Basic functions of lyssavirus proteins

N proteins encapsidate the genomic and antigenomic RNA. The resulting ribonucleoprotein (RNP) complex protects the RNA from degradation by RNAses. The phosphoprotein has at least two functions: it is the non-catalytic subunit of the polymerase complex, providing the connection between the RNP and viral polymerase protein, and antagonizes innate immunity in the infected host. The matrix protein coats the inside of the viral envelope, bridging the carboxy-terminal region of the glycoprotein and the RNP. Importantly, it sequesters the glycoprotein in a concentrated area of the cell membrane to aid in viral budding, although the role of the cytoplasmic region of the glycoprotein has been challenged by the finding that foreign glycoproteins with heterologous cytoplasmic sequences are well-incorporated into the budding virion[39,165,166]. The glycoprotein is a transmembrane protein which, in a trimer formation, constitutes the sole protein on the outer surface of the rabies virus (RABV) virion. The glycoprotein interacts with a host cell receptor and mediates pH-triggered fusion between the viral and host membranes, which results in the release of the RNP into the host cytoplasm. The glycoprotein is also the primary target for virus-neutralizing antibodies, which are necessary for protection against rabies disease. A unique feature of the rhabdoviral glycoprotein is that it can revert to its prefusion conformation once a neutral pH is restored[167], unlike the permanent fusion conformation exhibited by other viral proteins. The fusion mechanism of the closely-related vesicular stomatitis virus (VSV) has recently been elucidated[168]; however a lack of structural information limits our full understanding of the glycoprotein of RABV. RABV encodes its own RNA-dependent RNA polymerase because mammalian cells are not equipped to transcribe negative-stranded RNA. Moreover and in contrast with plus-stranded RNA viruses, neither the RABV genome nor anti-genome are functional templates for the viral polymerase unless encapsidated by the nucleoprotein. Therefore, the virus requires its own polymerase to perform the primary transcription of mRNA from the anti-genomic RNP and initiate the viral life cycle within the infected cell. After a threshold of transcription has been achieved and a certain amount of viral proteins has been produced, the polymerase switches to replication mode in which it ignores stop and start signals used to produce single mRNAs and produces a single, positive-stranded encapsidated plus-sense RNA. This anti-genomic RNA serves as a template for more copies of the genomic viral RNA. Such newly produced RNP are assembled together with the matrix protein and the glycoprotein into new virions at the cell membrane.

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Box 3:

Rabies virus as a model system for epidemiology and evolution

Mathematical models are invaluable tools to understand the mechanisms underlying contemporary and historical patterns of infectious disease transmission and to quantify the potential effectiveness of interventions for prevention and control[83]. The distinctive symptoms of rabies virus (RABV) and its global importance for human and animal health contributed to the early development of surveillance systems that recorded dates and geographic locations of incidence or exposure, information about the host and results from laboratory diagnostics. This unusual wealth of data for a non-human disease, often spanning decades and thousands of observations, has made rabies an exceptional system for modeling zoonotic infectious disease dynamics that has delivered applied and theoretical advances[169]. Foundational epidemiological models of rabies used a deterministic 'SEI' model, in which individuals were classified as susceptible (S), infected and incubating RABV ('exposed' (E)) or infected and infectious (I) with transmission governed by host density[86,170]. This simple model explained the observed epidemiological cycles for RABV infections in foxes and guided eventual strategies for elimination from Western Europe[171]. Since then, increasingly sophisticated models have captured diverse epidemiological features of RABV in domestic and wild reservoirs, including seasonality[118], individual heterogeneity in infectiousness[84], spatial dynamics[111], and the role of naturally acquired immunity among many others[172]. Similar modeling frameworks have been applied more recently to understand the dynamics of RABV infections in bats[85,97]. A critical application of models is in areas where rabies has not yet spread. For example, a recent model for the spread of canine rabies in Australia (currently RABV-free) highlighted geographic regions where research was needed to define parameters governing dog movements and behavior to prepare for the possible introduction of canine RABV[173,174]. Similar models of the spread of RABV in raccoons in Ohio, USA, provided rapid, high resolution recommendations for localities requiring vaccine delivery and enhanced surveillance efforts following a breach in a vaccine barrier[175].

The increasing availability of sequence data from RABV isolates is now driving the development of models that combine epidemiological and evolutionary data to better understand RABV transmission. RABVs are a reliable system for these models due to a combination of factors: its small single-stranded, non-segmented genome and its evolution on epidemiological timescales by point mutation (that is, without recombination or strong antigenic selection) enable for relatively simple evolutionary models; and its transmission dynamics (direct transmission, single species reservoirs, lack of arthropod vectors) remove much of the complexity typical to animal diseases. Consequently, RABV has been at the forefront of the development of statistical models in spatial phylogeography[176–178] and molecular inference of cross-species transmission[116,179]. Second generation sequencing is now expanding the depth and size of RABV molecular datasets. These data are providing insights into rabies transmission and evolution at unprecedented spatial and temporal scales[180]. The ability to characterize sub-consensus viral populations has enabled researchers to understand how genetic

diversity in local reservoirs contributes to host shifts to novel species[132]. Mechanistic models that link phylogenomic or sub-consensus distributions of sequence variants are now needed to fully exploit this new generation of data.

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- With much of the molecular virology characterized, we are now gaining appreciation of how nuanced rabies virus infection dynamics are and how immune status relates to transmission.
- The growing number of recognized rabies-virus related lyssaviruses highlights shortcomings in our discriminatory diagnostics and raises questions about their impact on human health. The lack of therapeutics for some of these lyssaviruses is a major concern.
- Enzootic maintenance of rabies virus within a population depends on transmission within narrow windows. The virus avoids extinction by both general and reservoir host-specific mechanisms with remarkable epidemiological plasticity.
- Features of rabies biology, ecology and evolution have made rabies a model pathogen for disease ecology and evolution. Recent work using mathematical models and phylodynamic analyses have allowd reconstruction and forecasting of epizootic rabies spread at the landscape level.
- At an evolutionary level, there is evidence that strong barriers prevent RABV from establishing in new host species, and both ecological opportunities and viral adaptation are needed to overcome these barriers. Studies of rabies virus molecular evolutionary dynamics have revealed the role of purifying selection and the importance of viral and host genetic backgrounds in host shifts. Further study of contemporary host shifts will likely shed light on rabies' unknown origin.
- We suggest that efforts be put into developing strategies and technologies to manage rabies viruses within bat reservoirs as has been previously done with carnivores, integrate diagnostics that can rapidly differentiate strains into surveillance efforts, hone our predictive power to detect outbreaks, and coordinate local resources to halt the spread of this lethal zoonosis.

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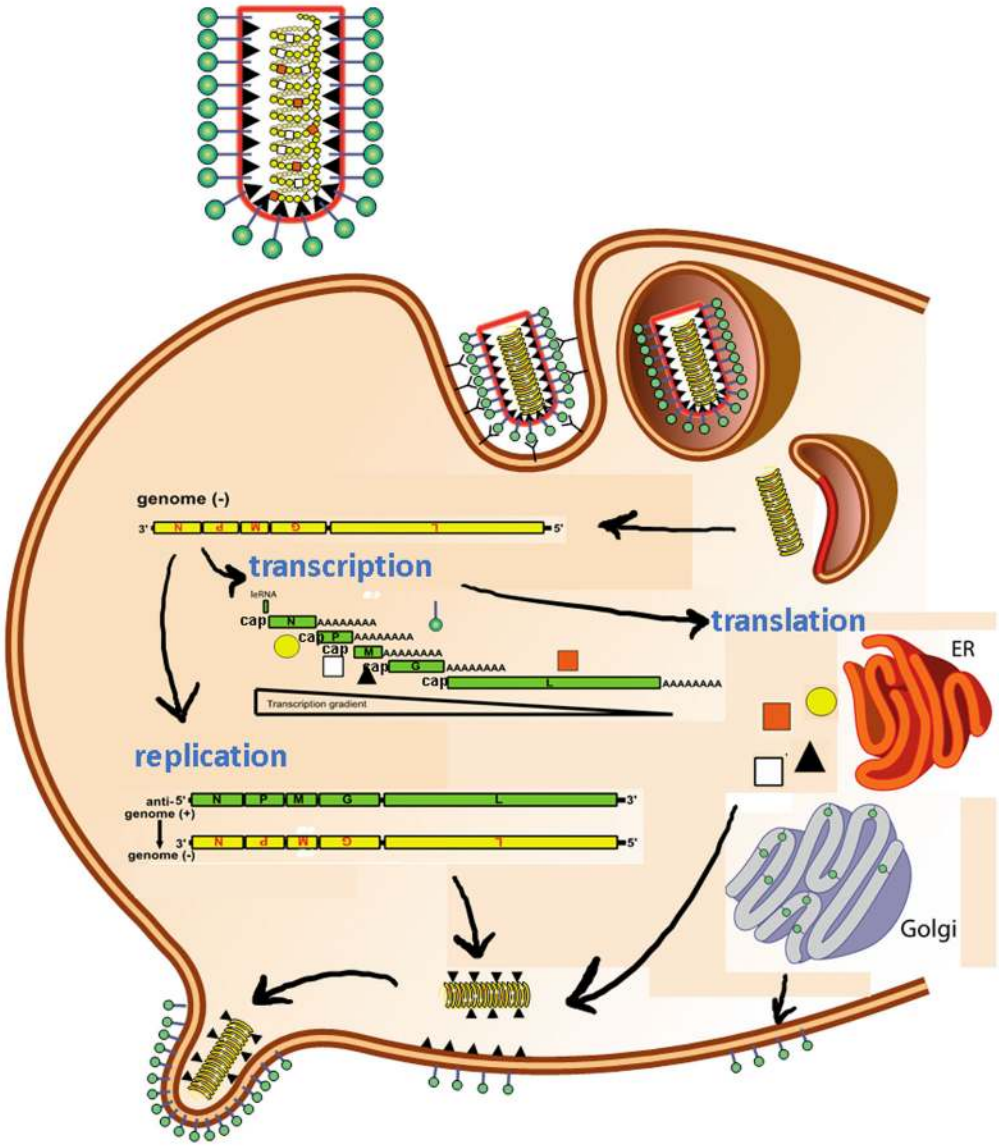

Figure 1a: Cellular life cycle of rabies virus.

In the first phase of the rabies virus (RABV) life cycle, the virus binds to the cell surface receptors via its glycoprotein and enters by endocytosis (step 1). Subsequently, the viral membrane fuses with the endosomal membrane to release the viral genome (uncoating, step 2). In the second phase, the encapsidated negative-stranded RNA genome is transcribed by the polymerase complex, starting with a short uncapped leader RNA (leRNA), followed by the transcription of 5′ end-capped (cap) and polyadenylated (A) mRNAs, and their translation into the viral proteins nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L) (steps 3 and 4). Following replication, the full-length antigenomic RNA is encapsidated in the nucleoprotein protein along with the genomic RNA. The synthesized antigenome functions as a template for the synthesis of additional copies of genomic RNA (step 5). In the last phase, the viral components are assembled and the RABV virions bud and are released, starting a new round of infection (step 6).

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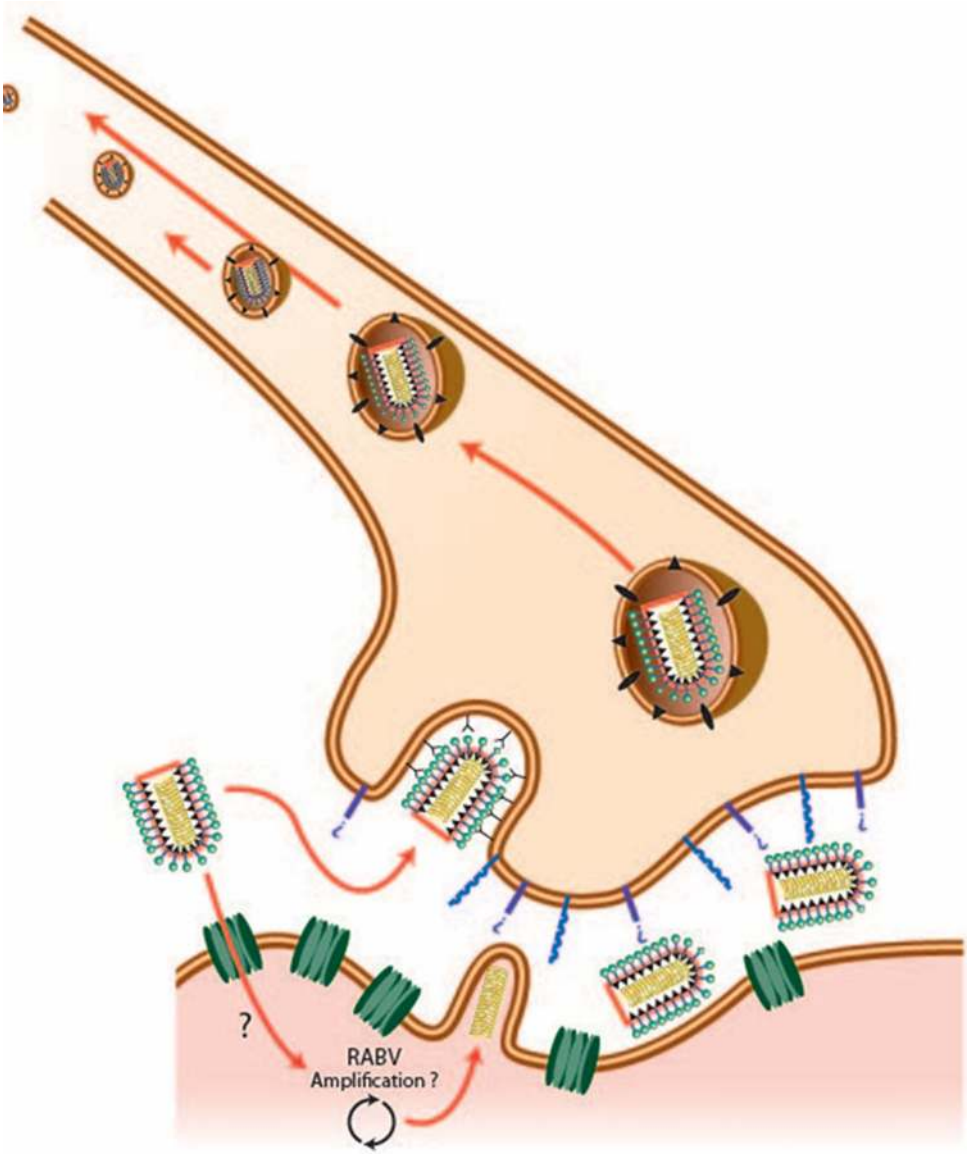

Figure 1b: RABV entry and transport in neurons.

The nicotinic acetylcholine receptor (nAchR) is located at the postsynaptic muscle membrane. It has been suggested that the nAchR receptor enriches RABV at the neuromuscular junction (NMJ, synaptic cleft), enabling more efficient infection of the connected motor neurons. Other research suggests that initial virus amplification occurs in muscle (indicated by the question mark), which indicates that nAchRs might be used to infect muscle cells. RABV can enter neurons by binding to NCAM or another, unknown receptor. Following uptake by clathrin-mediated endocytosis, RABV virions are then transported within the vesicle and are released in the cell body of the infected neurons, where replication and transcription occurs (not shown). Parts a and b modified from Ref 34

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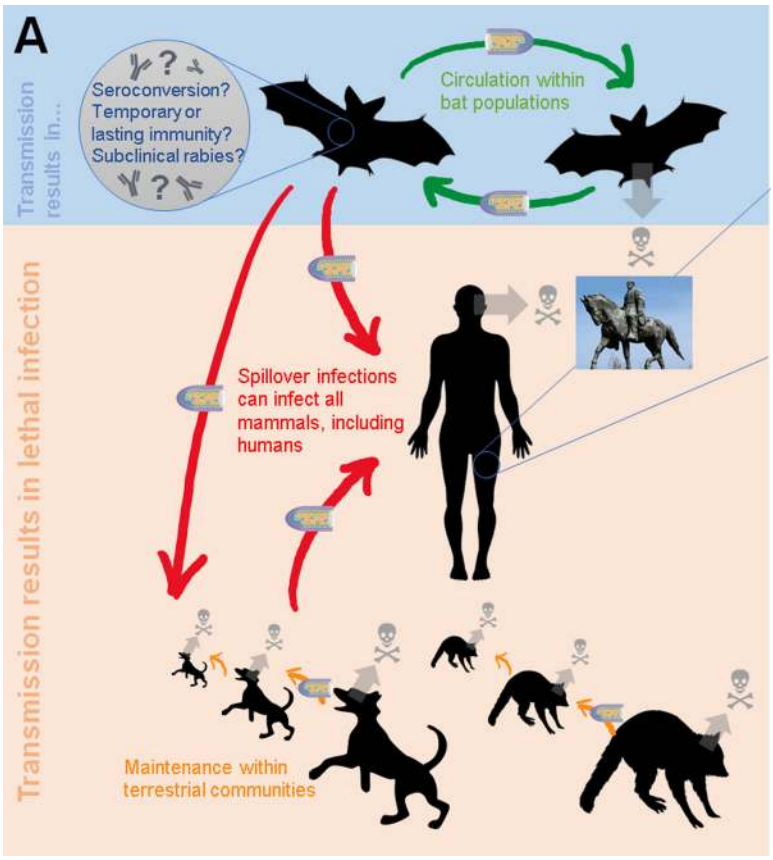

Figure 2: Lyssavirus transmission dynamics in bats and terrestrial animals.

Lyssaviruses seem to circulate more successfully in bats than in terrestrial mammals. A variety of factors contribute to maintenance, such as general and reservoir-specific viral factors, habitat factors and levels of human interaction. Host-specific factors, especially immune status in bats, are less understood. Although bats die upon clinical manifestation of disease, high rates of seroprevalence seen in healthy bats suggests a high frequency of abortive infection, which occurs through a combination of low exposure dosages, functional innate immune, and presence or development of neutralizing antibodies. Experimental infection studies have shown that bats can develop sub-detectable immunological memory (protection in the absence of neutralizing antibodies). However, there has never been evidence to suggest that a healthy but virus-secreting “carrier” state contributes to maintenance within bat populations. Seroprevalence in non-bats arising from natural exposure (not vaccination) are lower by comparison. Maintenance of RABV in wild and domestic carnivore reservoirs of RABV depends more heavily on variation in life history traits, population density, habitat use and degree of associations with humans. Spillover infections (in humans, for example) almost exclusively result in death before transmission to another host. The biological factors underpinning whether hosts survive or succumb to rabies, if naturally acquired VNAs are protective, and what role this has in the long-term perpetuation of RABV remain unclear.

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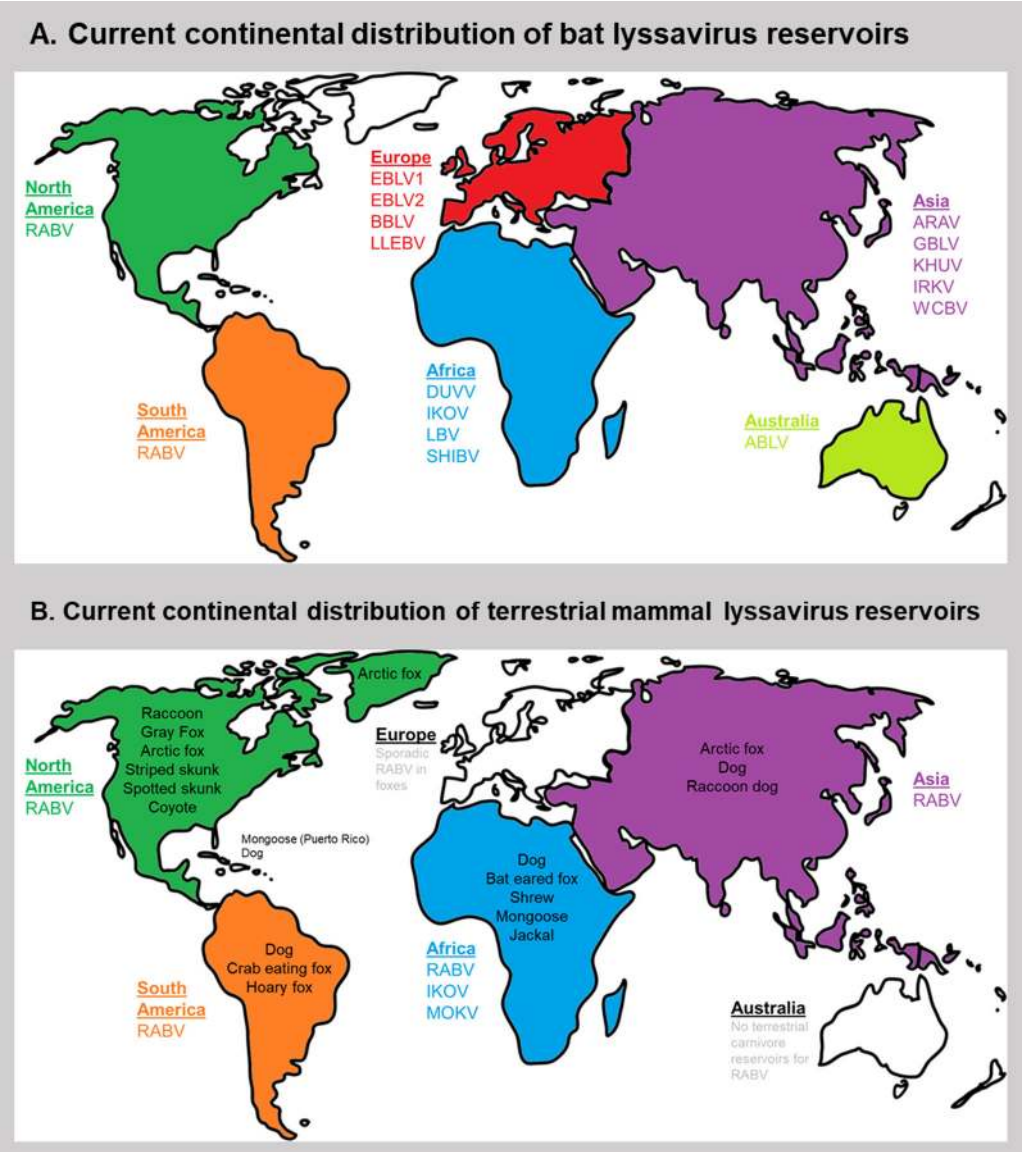

Figure 3A: Current continental distribution of bat lyssaviruses.

Rabies disease-causing lyssaviruses circulate on all continents except Antarctica. In the Americas, all variants to date are strains of RABV. By contrast, RABV is largely absent from bats in the rest of the world, but 14 other lyssaviruses have been found in bats, including in Australia and Europe which are otherwise considered RABV-free.

3B: Current continental distribution of terrestrial mammal lyssavirus reservoirs. Most terrestrial lyssavirus reservoirs circulate strains of RABV, except in Africa, where IKOV and MOKV have been found. Europe is considered free of terrestrial RABV on account of vaccination efforts. Rabies in terrestrial mammals has never been reported in Australia. Common reservoirs are indicated.

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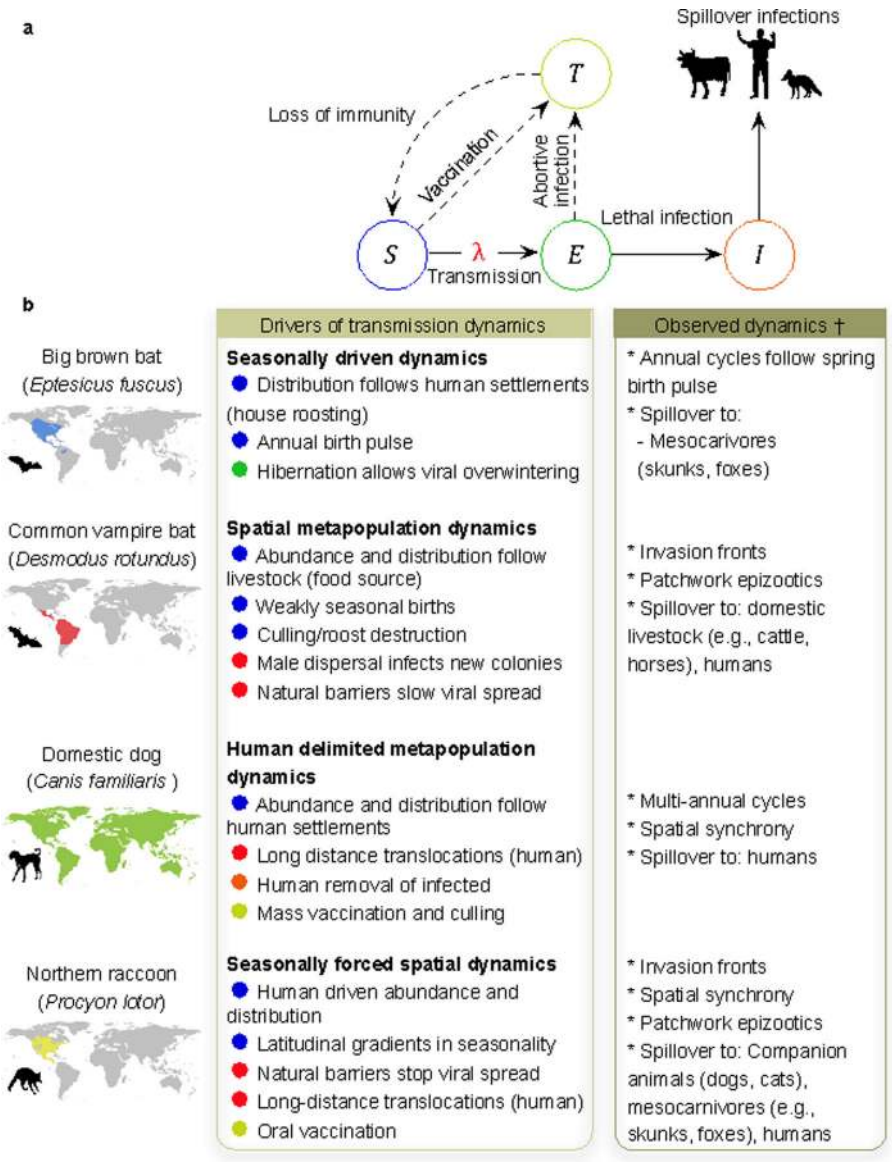

Figure 4: The influence of reservoir host ecology on the epidemiology of rabies.

a| The 'SEI' model for rabies epidemiology represents transitions of hosts between individuals that are classified as susceptible (S), infected and incubating RABV ('exposed' (E), infected and infectious (I) and temporarily immune (T). Both intraspecific transmission and spillover infections to non-reservoir hosts arise from infectious reservoirs in the I class. The force of infection (λ) depends on the frequency of susceptible and infected and infectious individuals in the population, total population size and transmission rate. Solid arrows indicate portions of the transmission cycle that are common to all RABV reservoirs. Dashed arrows may only occur for some reservoir hosts, depending on pathbiological relationships with rabies (see Fig. 2) or human interventions such as the presence of vaccination campaigns. **b|** Variation in reservoir host ecology influences different phases of the transmission cycle, causing reservoir host-specific transmission dynamics and maintenance mechanisms. Maps show the geographic range of four of the best-studied

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RABV reservoir hosts, although RABV may be absent from some parts of each species' range, for example because of changing viral distributions or local eradication efforts. The drivers of transmission dynamics are mapped for each reservoir host to the compartmental model in panel **a**, to illustrate how ecology affects transmission dynamics. The epidemiological dynamics observed in each reservoir are summarized.
Of note, spillover infections to a broad range of species occur from each reservoir; shown are the principle spillover hosts infected by each reservoir.