# Lyssaviruses and the Evolution of Rabies:

# A Riddle Wrapped in an Enigma[1]

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[1] This paper will be updated based on feedback which has been sought from various authorities more intimately connected with the field: please check http://uc.edu.kh/ucs/UC%20Publications/8/2763/ for the latest version.

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**Abstract**

Rabies is an important cause of human mortalities, with human cases in the developing world being mainly due to spill-overs of the rabies virus from dogs as the natural reservoir; such a source has been largely eliminated in developed countries, where bats constitute a major residual source of spill-overs. In addition, bats would appear to be the reservoir for many, if not all, other members of the genus *Lyssavirus* in the Old World, some of which may spill over into humans on occassion. Where known, the rabies and related viruses are transmitted in the saliva of an infected animal to the muscles of a potential new host by biting; thereafter, the virus spreads into and within the central nervous system and thenceforth output targets (most especially the salivary glands) for further onward transmission; this is maximised in the case of 'furious' rabies, where the virus essentially commandeers the brain towards its own ends. After reviewing current knowledge regarding the diversity of recognised species within the genus *Lyssavirus*, the nature of typical rabies infections in terrestrial mammals is reviewed, together with evidence for atypical *in vivo* investigations, in the laboratory and in the wild. Thereafter, the riddle of the nature of the effects of natural and experimental infections of rabies and other lyssaviruses in bats is reviewed. Finally, given the assumption that lyssaviruses originated from spill-over(s) of an ancestral rhabdovirus from insects into bats, consideration is given as to the enigma of how the sophisticated highly-derived cycle of the rabies virus in terrestrial mammals may have originated, and how this might also resolve the riddle presented by bats.

## I. Introduction

Rabies is a dreadful – and dreaded, for those in the know – disease, typically transmitted by bites from infected dogs (Schnell *et al.*, 2010), being caused by the type species of the genus *Lyssavirus* (family Rhabdoviridae). Nevertheless, rabies is a largely underestimated disease (Hampson *et al.*, 2015). Thus, globally, spill-overs from dogs kill more people than either yellow fever, dengue fever or Japanese encephalitis (Knobel *et al*., 2007); in Cambodia, there are an estimated 800 to 900 cases each year, as compared with the 56 human cases of avian flu over the eleven years up until 2014 (Amaro, 2016).

The rabies virus is the most lethal microbe known: death is virtually certain in those infected once the virus has invaded the central nervous system (Jackson, 2008, 2014; Hemachudha *et al.*, 2013). Documentary evidence suggests that an association with infections from rabid dogs

can be traced back to at least 4,000 years ago in Mesopotamia, together with reports in other early texts from India and China as well as, later, ancient Greece and Rome (Tarantola, 2017). Tarantola (2017) has documented the various 'cures' which have been tried: until the development of a vaccination strategy by Louis Pasteur in the early 1880s, perhaps the most merciful was to smother the afflicted person with mattresses to alleviate (albeit terminally) the individual's suffering. Post-exposure prophylaxis, the procedure which Pasteur pioneered, remains (with various refinements) the only method for pre-empting infection after being bitten by a dog or other animal which is rabid (Johnson *et al.*, 2010). Much more effective is to remove the risk of such bites in the first place through active measures to control the circulation of the virus and related *Lyssavirus* spp. (at least some of which can also spill over into humans) in existing animal reservoirs (Knobel *et al.*, 2013; Tarantola, 2017).

Rhabdovirids are members of the order Mononegavirales: as such, their genetic material is a single-stranded negative-sense RNA molecule. The family would appear to have originated in arthropods (Dietzgen *et al*., 2012; Longdon *et al.*, 2015) and today comprises a wide diversity of different genera, including some which circulate exclusively in arthropods and others which use these as vectors to cycle between plants or between vertebrates (Kuzmin *et al*., 2009; Rupprecht *et al.*, 2011). On the other hand, lyssaviruses are generally considered to be enzootic to mammals, possibly originating from an ancestral spill-over from insect prey which was able to adapt to and thereafter adopt early insectivorous mammals as their sole hosts (Badrane and Tordo, 2001; Longdon *et al.*, 2015); alternatively, they may have been acquired from haematophagous arthropod vectors (Constantine, 2009).

In targeting the nervous system, the rabies virus adopts a strategy which has similarities with that of 'heirloom' human herpes viruses. However, as will be considered further below, there are notable differences, the most prominent of which is that rabies takes over control of a significant portion of the central nervous system; in this way, it causes changes in behaviour which are most obvious in the case of 'furious' rabies where the virus may be transmitted in saliva to new hosts before the present one dies as a result of infection. This 'active' form of onward transmission contrasts with the passive 'sit-and-wait' strategy seen with latent infections of sensory neurones by herpes-related viruses in humans, for example; and it raises questions about how such a sophisticated, highly derived state could have evolved from a virus originally circulating in arthropods.

The following sections will briefly review some aspects of the biology of lyssavirus infections, as a background to speculating how they might have evolved their present transmission

strategy. Thus the diversity within the genus *Lyssavirus* will be considered first, followed by a review of salient features regarding infections in terrestrial mammals as the preliminary to a section considering the riddle of various aspects related to infections of rabies and other lyssaviruses in bats. Thereafter the final section will discuss the enigma of how the present-day highly derived state in bats and other mammals might possibly have evolved from what was originally an arthropod virus.

## II. The Genus *Lyssavirus*

Rhabdovirids derive their family name from the characteristic bullet-shape of their virions in electron microscope studies: the nucleoprotein-associated single negative strand of RNA is enclosed within a proteinaceous capsid which is in turn enveloped by a membrane (derived from the host cell) which incorporates a viral glycoprotein responsible for mediating infection of another target cell after the virion has been shed (Schnell *et al.*, 2010). Associated with the nucleocapsid are RNA-dependent RNA polymerase (RdRp) molecules, responsible for the initial transcription of positive RNA strands upon infection of the new host cell; however this process is prone to errors due to the lack of any proof-reading activity, meaning that mutations are relatively more frequent than in DNA-based systems (*e.g.* Holmes, 2003, 2008; Belshaw *et al*., 2008). If a virus is already optimally adapted to its present host, such mutations are likely to be to the detriment of its progeny; on the other hand, they may be advantageous in ongoing evolutionary arms races with their existing host-reservoir (through variation in the latter's innate immune system together with recently-developed targeted therapies), as well as opening up opportunities to spill over into new reservoirs (reviewed by *e.g.* Munro, 2017).

Different members of the genus have been identified based on analyses of their RNA sequences. Such genomic analyses have been used not only to identify the likely phyletic relationships between the different species but also have included 'molecular clock' approaches to try to determine time-lines for divergence from their most recent common ancestors. However interpretation of the latter results needs to be considered with a degree of scepticism, in rhabdovirids (Kuzmin *et al.*, 2009; Velasco-Villa *et al.*, 2017) as in other viruses. Thus, available viral sequences give only a brief snapshot of changes over time (typically within the past half-century or so, at most), and are often restricted in space; they reflect the recent evolution of a presumably much older lineage against a background of mutations, the numbers of which are likely to be enhanced by the inherent inaccuracies in RdRp-mediated replication (Ho *et al.*, 2011; Wertheim and Pond, 2011; Wertheim *et al.*, 2013; Holmes, 2016; Rupprecht

*et al.*, 2017). Evolution over longer time-scales and in different locales serves to sort the wheat from the chafe: some mutations may be selected for (*e.g.* as part of an ongoing arms-race with the local reservoir hosts' innate immune and other defense systems) and hence form part of an overall evolutionary core 'thread' of sequences, whilst others will be weeded out along the way to be replaced by other mainly short-lived variants as a result of purifying selection and mainly neutral evolution in lyssaviruses (Holmes, 2008; Wertheim and Pond, 2011; Wertheim *et al.*, 2013; Mollentze *et al.*, 2014; Rupprecht *et al.*, 2017; Marston *et al.*, 2018).

## 1. Overall Diversity within the Genus

A total of 14 species have been officially recognised by the International Committee on Taxonomy of Viruses (ICTV), including the type species *Rabies virus*; two others have yet to be formally accepted.[3] Of these, all but two have been identified in bats (Banyard *et al.*, 2014a; Mackenzie *et al.*, 2016; Banyard and Fooks, 2017). Thus it has generally been assumed that the genus *Lyssavirus* originated in bats, and that this took place in Africa (*e.g.* Rupprecht *et al.*, 2017). However this geographical origin has been questioned (Kuzmin and Rupprecht, 2007), and a recent phylogenomic analysis by Hayman *et al*. (2016) concluded that the presumed original spill-over from arthropods into bats occurred in Palaeoarctic Eurasia; thereafter, three separate onward spillings-over within mammals led to the genus' outward radiation into Africa, each associated with a major clade (phylogroups I-III).[4]

Apart from the rabies virus, phylogroup I includes nine other species (Hayman *et al*., 2016; Mackenzie *et al.*, 2016), including two yet to be officially recognised as such; all have been associated with bats. Most closely related are the Gannoruwa bat lyssavirus (provisionally identified in fruit-bats from Sri Lanka: Gunawardena *et al*., 2016) and the Australian bat lyssavirus (from fruit-bats and one species of insectivorous one: Banyard *et al.*, 2011, 2014a); in addition, there are three other lineages, with the relatively abundant (presumably because of sampling bias) European bat lyssavirus types 1 and 2 (from insectivorous bats, typically being associated with the vespertilionids *Eptesicus* spp. and *Myotis* spp., respectively: McElhinney *et al.*, 2013) belonging to different ones. The Duvenhage virus, in the same lineage as European bat lyssavirus type 1, is the only one to have been found outside Eurasia, having been isolated

[3] https://talk.ictvonline.org/taxonomy/ (2016 release); Amarasinghe *et al*. (2017).

[4] A. D. Munro (in prep.) has speculated that Southeast Asia may have been an important source of lyssaviruses, given the serological evidence for past infection of a diversity of bat species, despite the general failure to identify any current infections.

from two insectivorous bats – *Miniopterus schreibersii*[5] and *Nycteris thebaica* – in southern Africa (van Eeden *et al.*, 2011) and from humans infected there (van Thiel *et al*., 2009).

Phylogroups II and III each comprise three recognised species (Hayman *et al*., 2016; Mackenzie *et al.*, 2016), only some of which have been associated with bats.[6] The former is restricted to Africa and includes the Lagos bat virus (from various species of fruit-bats in western and southern Africa: Markotter *et al.*, 2008; Kuzmin *et al*., 2010)[7] and the Shimoni bat virus (from an insectivorous bat, *Hipposideros vittatus*, in Kenya: Kuzmin *et al*., 2010). On the other hand, the Mokola virus was isolated from shrews (*Crocidura* sp.)[8] in Nigeria; it has since been found in southern Africa in other species (most especially in domestic cats), including humans, with clinical features of a rabies-like infection (Kgaladi *et al*., 2013; Mackenzie *et al.*, 2016; Coertse *et al.*, 2017), but not from any bats (although there is serological evidence for past infections in some fruit-bats: Dzikwi *et al*., 2010).

On the other hand, phylogroup III is represented by two Eurasian species from *Mi. schreibersii*, the West Caucasian bat virus (from southern Russia, near the border with Georgia: Kuzmin *et al.*, 2008a)[9] and the Lleida virus (from Spain: Ceballos *et al*., 2013); together with an African one, the Ikoma virus, isolated from a civet (*Civettictis civetta*)[10] with clinical rabies in a dog-free portion of the Serengeti National Park in Tanzania, there being no evidence for infection of bats in the area (Horton *et al*., 2014).

## 2. Strain Diversity within *Rabies virus*

Velasco-Villa *et al.* (2017) distinguished between strains (biotypes)[11] of this species which have evidently circulated for a prolonged period within an existing reservoir host species (be

[5] The species identification was tentative; moreover, if this was correct, then a recent taxonomic analysis suggests that it should instead be assigned to *Mi. natalensis* (Miller-Butterworth *et al.*, 2003).

[6] Note that phyllogroup III is based only on genomic studies, without any serological or other evidence for an underlying unitary nature (Rupprecht *et al*., 2017).

[7] With evident dead-end spill-overs into a rabid carnivoran, the marsh mongoose (*Atilax paludinosus*), in South Africa (Markotter *et al*., 2006) and into cats and dogs (Banyard *et al*., 2011); the Lagos bat virus is likely to be paraphyletic, with at least four component clades, A-D (Banyard *et al*., 2011; Freuling *et al*., 2015).

[8] Also insectivorous; consistent with the proposed ancestry of rhabdoviruses in general, this virus is also able to replicate in insects and in insect cells (Buckley, 1975; Aitken *et al.*, 1984).

[9] With serological evidence for this or a related species in *Miniopterus* spp. in East Africa (Kuzmin *et al*., 2008d).

[10] A viverrine carnivoran, suggested to be an unlikely reservoir; those species of bat sampled in the vicinity were seronegative.

[11] One definition (Wandeler, 2004) is that "[a] particular biotype is a virus variant adapted to a particular principal host species by especially high pathogenicity for this species, by a high rate of excretion, and by low immunogenicity."

they dogs or particular species of bat) and those which have more recently spilled over to become established and circulate within new reservoir host species: respectively designated as 'maintained' and 'derived' viral strains. Although the rabies virus is capable of infecting virtually all eutherian mammals (including rodents, 'ungulates'[12] and humans), natural reservoirs of rabies viruses have been identified in only two of the 29 mammalian orders: Carnivora and Chiroptera, each with their own circulating strains (Hanlon *et al.*, 2013). Within the Carnivora, this is in three of nine families in the suborder Caniformia – the Canidae (dogs, foxes, raccoon-dogs, *etc.*), Mephitidae (skunks) and Procyonidae (racoons) – and in one of the five families, the Herpestidae (mongooses), in the suborder Feliformia (Hanlon *et al.*, 2013): whilst domestic cats (*Felis silvestris catus*; family Felidae) are an important source of human infections, they themselves are relatively resistant to rabies and likely serve as bridging hosts mediating spill-overs from other carnivorans or bats rather than being a natural reservoir (Constantine, 2009). Concerning bats, rabies virus has been identified in a number of different families – most especially vespertillionids, mollosids and phyllostomids (including vampire bats) – in the Americas (Constantine, 2009; Escobar *et al.*, 2015).

Genomic analyses have been used to characterise the different strains of the rabies virus, with the identification of six clades in dogs (Smith *et al.*, 1992). Overall evidence indicates that their evolution has been tightly constrained: there is limited evidence for the positive selection of non-synonymous mutations, and hence for potential adaptive evolution, when strains in the carnivoran and bat orders were considered together (Holmes *et al.*, 2002), although a subsequent more extensive survey restricted to bat rabies virus strains in the Americas led to the identification of additional evidence for phylogenetically 'local' (intra-ordinal) positive selection, most especially in the ectodomains of the glycoprotein, which was related to the episodic establishment of lineages in new host species of bats (Streicker *et al*., 2012a). This is in contrast to the often large positively-selected (non-synonymous) variability in the genes for surface glycoproteins in other viruses, as a means not only to evade the immune system but also to diversify the range of host acceptor molecules with which they can interact and thus infect (potentially new) target cells. Holmes *et al.* (2002) suggested that it might reflect the constraints imposed on the rabies virus by the need to replicate in different tissues (neurones, salivary gland cells and presumably muscle cells, at least; see Section III) within its present host; or, alternatively, it was the result of immune selection, due to the virus' active measures to limit the subsequent development of an immune response upon infection (see Sections III.2

[12] Comprising members of the orders (Cet-)Artiodactyla and Perissodactyla.

and V.1). As a result, the prospective host, amongst 'typical' terrestrial carnivoran hosts at least, normally does not mount an immune response to the initial infection, but only in the terminal stages (see Section III.5).

The following sections will briefly review the prevailing situation regarding rabies infections in the Old and then the New Worlds.

***i. Old World Rabies Viruses*** Infections would seem to be primarily associated with strains maintained in dogs (*Canis lupus familiaris*) as a reservoir today, with evidence for strong purifying selection (Troupin *et al.*, 2016). Thus various regional clades have been identified based on genomic analyses of viruses from rabid dogs (recently reviewed by Troupin *et al.*, 2016; Munro, 2017; Velasco-Villa *et al.*, 2017); the largest number of these is found in Asia, with phylogenomic data suggesting that the species may have originated in the Indian subcontinent (Bourhy *et al.*, 2008)[13] or in East Asia (Meng *et al.*, 2011).[14] On the other hand, the most widespread clade today is the Cosmopolitan (including the archetypal genotype 1 Pasteur), which is found in dogs in Europe, together with Africa, and western portions of Asia as well as in the Americas.

Whilst reports of rabies in dogs and the consequent infection of humans date back about four millennia, the Cosmopolitan clade appears to have originated when urban rabies emerged as a threat in Europe in the first half of the 18th century when spill-overs of sylvatic rabies from an unknown reservoir became established in the growing population of dogs associated with humans (Blancou, 2004; Nadine-Davis and Bingham, 2004). This strain presumably outcompeted previously existing strains, accounting for the incongruence with documented historic cases (Troupin *et al.*, 2016). A new biotype of this clade became established in European red foxes (*Vulpes vulpes*) during the twentieth century in what is now Russia, to subsequently spread westwards, including *via* an alternative host, raccoon dogs (*Nyctereutes procyonoides*; escapees of stocks introduced from East Asia for the fur-trade) as a presumed spill-over rather than the original source (Bourhy *et al.*, 1999; Wandeler, 2004).

[13] It is interesting that the Gannoruwa bat lyssavirus isolated from fruit-bats (*Pteropus medius*) in Sri Lanka is the most closely-related known species of lyssavirus to the rabies virus (Gunawardena *et al*., 2016), recalling the proposal of Bourhy *et al.* (2008) that rabies originally spilled over into dogs on the Indian subcontinent.

[14] Exemplifying the problems associated with reconciling these and molecular clock analyses, Troupin *et al.* (2016) concluded that the last common ancestor of *all* dog-related rabies viruses dated back to between 1308 and 1510, with earlier reports of rabies infections being caused by other strains which have since died out.

In recent times, the Cosmopolitan clade has spread into sub-Saharan Africa with the introduction of infected dogs during the colonial period, apparently displacing pre-existing strains apart from in West Africa (Nel and Rupprecht, 2007;[15] Velasco-Villa *et al.*, 2017); spill-overs have thence established variants circulating in populations of wild canids, for example, although an antecedent diverse biotype is still circulating in present-day African mongooses (Herpestidae) and civets (Viverridae) in southern Africa.

Another, the Arctic clade, is relatively closely related to the Cosmopolitan one and has been identified in European red foxes and arctic foxes (*V.* [= *Alopex*] *lagopus*), with the latter as the major reservoir in northern Asia, as well as in Alaska, Canada and Greenland (Mørk and Prestrud, 2004; Nadine-Davis and Bingham, 2004; Kuzmin *et al.*, 2008b; Nadin-Davis *et al.*, 2012; Goldsmith *et al.*, 2016), wherein there are cyclical eruptions which may spill over into sled-dogs (Raundrup *et al.*, 2015); it may have recently spread northwards to infect these canids from dogs infected with 'Arctic-like' members of this clade from as far south as Iran and the Indian subcontinent (Bourhy *et al.*, 2008; Velasco-Villa *et al.*, 2017).

Apart from one unusual report from southern China (Lu *et al*., 2013; see Section IV.1), the rabies virus itself has not been identified as such in bats from the Old World; contamination of laboratory samples is likely to have been a problem in other isolated cases (Banyard *et al*., 2011, 2014a; Barrett, 2011; Kuzmin *et al*., 2011). Instead other species have been isolated (see previous Section).

***ii. Rabies Viruses in the Americas*** Three major groupings of these have been identified as circulating in the New World. One, the Arctic clade (see above), has been found to spill over from their reservoir hosts into dogs, but has only made limited incursions further south; this, together with evidence for a recent origin based on genomic analyses (the usual caveats apply), led Velasco-Villa *et al.* (2017) to conclude that it has not contributed to the present-day viruses circulating elsewhere in the Americas.

The second group comprises members of the Cosmopolitan clade. Outbreaks of urban rabies were first reported in North American cities on the east coast in the latter half of the eighteenth century, to spread westwards thereafter (Nadine-Davis and Bingham, 2004; Velasco-Villa *et al.*, 2017). Genomic analyses of recent viruses indicate that this is likely to have been due to the importation of the strain from Europe (Velasco-Villa *et al.*, 2017); the delay in its becoming established in North America may be partly as a result of the initially long trans-Atlantic

[15] Note that their 'horn of Africa' refers to West Africa.

crossing times, such that these exceeded the typical latencies for rabies infections to become manifest in dogs so that the latter would have been eliminated *en route* (Velasco-Villa *et al.*, 2017; *cf.* Troupin *et al.*, 2016). Prior to this, cases of evident rabies in dogs may have been imported ones (presumably with an atypically long latency) or spill-overs of pre-existing indigenous American strains from sylvatic hosts (see below) which failed to become established due to the low population densities of dogs at the time (Nadine-Davis and Bingham, 2004; Velasco-Villa *et al.*, 2017).

Arising out of the original introduction of the Cosmopolitan clade into North America, there have been derived spillings-over not only into other canids (including coyotes, *Canis latrans*) but also into certain populations of skunks (*Mephitis* spp.) (Nadine-Davis and Bingham, 2004; Velasco-Villa *et al.*, 2017).

A third cluster is represented by the indigenous American clade (Banyard *et al.*, 2011, 2014; Rupprecht *et al.*, 2011), which is distinct from the 'dog-related' clades of *Rabies virus* (Troupin *et al.*, 2016). This has been proposed to have arisen as a result of the 'reverse' spill-over from infected dogs to common vampire bats (*Desmodus rotundus*)[16] in Central and South America, through the latter's ingestion of infected blood, wherein a new strain evolved as an enzootic; thereafter, this strain is argued to have spilled over *via* a transmission chain into other species of bat,[17,18] leading to the emergence of other biotypes of virus, thereby explaining why the rabies virus has been very rarely been identified in Old World bats. The spread of infection would possibly have been facilitated by the population explosion of vampire bats as a result of the "massive" introduction of livestock in the latter half of the 17th century (Rupprecht *et al.*, 2011), with dispersal being aided by the migratory nature of many other species of bat. As a result, rabies from common vampire bats is a major cause of livestock losses in Central and South America (Lord, 1980; Johnson *et al*., 2014), whilst insectivorous bats are a major source of human rabies cases in North America now that dogs have been eliminated as a reservoir

[16] Members of the Phyllostomidae, a diverse group of New World bats which includes not only these but also insectivores and species which feed on fish and other small vertebrates, nectar or pollen.

[17] As well as from these into striped skunks (*M. mephitis*), distinct from dog-derived ones (Troupin *et al.*, 2016).

[18] Including two other species of vampire bat which feed predominately on blood from birds (Fenton, 1992; Naish, 2007a; Constantine, 2009); a shared rabies virus strain has been identified in the hairy-legged vampire bat, *Diphylla ecaudata* (Castilho *et al*., 2010) and in the white-winged vampire bat, *Diaemus youngi* (de Thoisy *et al*., 2016). Whilst birds may be infected with standard rabies viruses experimentally, there are only rare reports of (presumably dead-end) transmission of dog-related strains under natural circumstances (Baby *et al.*, 2015).

(Lackay *et al.* 2008; Hanlon and Childs, 2013), including through the intermediary of cats, which are relatively resistant to rabies infections (Constantine, 2009).

However this scenario is open to question. In contrast to the debatable evidence based on 'molecular clock' analyses in support for such a scenario (Hughes *et al.*, 2005; Kuzmina *et al.*, 2013; Hayman *et al.*, 2016; Troupin *et al.*, 2016; Rupprecht *et al*., 2017; Velasco-Villa *et al.*, 2017), there is suggestive evidence that a rabies virus may have been enzootic prior to this time, especially in vampire bats. Thus although Spanish conquistadores were afflicted by the bites of vampire bats, the indigenous peoples had developed the practice of cauterising bites (Baer, 2007; Constantine, 2009). Whilst the invading colonialists were familiar with the more recognisable 'furious' manifestation of rabies (see Sections II.2.i and III.2) from outbreaks in Europe, the strain transmitted by vampire bats is mainly associated with development of the 'paralytic' form of disease;[19] thus early cases of rabies infection may have been misdiagnosed as being due to poliomyelitis or a related disease, with cattle suffering from 'plague' or 'botulinism'. Similarly, prior to the identification of rabies in an insectivorous bat in Florida in 1953, human spill-overs may have been misdiagnosed as being due to poliomyelitis in the United States (Constantine, 1967; Kuzmin and Rupprecht, 2007).

Regardless of its provenance, comparative analyses of the sequence of the glycoprotein gene indicate that there are four groups of strains within the indigenous American clade, more related to behavioural lifestyle of the host species than their geographic distribution or their phylogenetic relationships (Hughes *et al.*, 2005; Davis *et al.*, 2006; Velasco-Villa *et al.*, 2006; Banyard *et al.*, 2011). Group I is associated with colonial, non-migratory bats (*Myotis* spp. and *Eptesicus fuscus*; the latter also hosts group III viruses in western parts of its range) and group II with solitary,[20] migratory species (*Lasiurus* spp., *Lasionycteris noctivagans* and *Perimyotis* [= *Pipistrellus*] *subflavus*,[21] apparently being a later 'acquisition': Hughes *et al.*, 2005). On the other hand, group IV, which is basal to the other three groups, comprises *D. rotundus* (family Phyllostomidae) and subspecies of *Tadarida brasiliensis* (family Mollosidae), a colonial migratory bat found throughout much of the Americas (Davis *et al.*, 2006; Kuzmina *et al.*, 2013), as well as various non-haematophagous neotropical bats, with evidence for another two

[19] But see Lopez *et al.* (1992) for a recent outbreak in the Peruvian Amazon where a vampire bat strain of rabies was instead associated with outbreaks of the furious form of the disease in two isolated villages, perhaps related to the recent development of pig-farming (subsequently aborted).

[20] Note that these come together seasonally in mating and maternity roosts and, in some cases, to hibernate.

[21] Naish (2011a) has reviewed the taxonomy.

taxonomically- and geographically-based sub-groups (Oliveira *et al*., 2010; see also Banyard *et al*., 2011; Johnson *et al.*, 2014). However, whilst such analysis clearly distinguished members of the indigenous American clade from those circulating in terrestrial mammals in the Old World, similar comparisons for the nucleoprotein and phosphoprotein genes indicated that those derived from bats formed a discrete cluster nested within the inferred trees for rabies viruses as a whole. Thus Davis *et al.* (2006) concluded that it was impossible based on present-day samples to determine whether the rabies virus originated in bats or in terrestrial mammals, or simultaneously in both.

A genomic analyses of the rabies viruses of 23 North American bat species led Streicker *et al.* (2010) to conclude that most spill-overs were into closely-related host species, despite the intrinsic mutability of these RNA viruses. Moreover most spill-overs were evolutionary dead-ends, with successful establishment being dependent on the frequency of spill-overs between the two species involved. They suggested that the similarities between closely-related species in terms of various aspects of their biology were also important determinants: for example, virulence (with the need for the deleterious effects on the brain to be sufficiently delayed for there to be time for sufficient salivary excretion) is more likely to be already near the optimum based on similarities in *e.g.* cell-surface acceptors and other aspects of their biochemistry and physiology (Streicker *et al*., 2010). Streicker *et al*. (2012b) proposed that the main factor influencing the diversification of the rabies lyssavirus amongst New World bats was likely to be the opportunity for year-round transmission and infection in tropical populations, and thus more 'generations', possibly together with the increased chance of spill-overs to infect the greater diversity of other potential host species.

Moreover, whilst skunks in more northerly portions of the central United States and Canada are host to a derived Cosmopolitan strain, a bat-derived variant is circulating in these from more southerly regions, with evidence for multiple independent spill-over events, typically from *Ep. fuscus* (Rupprecht *et al.*, 2011; Kuzmin *et al.*, 2012; Velasco-Villa *et al.*, 2017). Similar spill-overs have also occurred into raccoons (*Procyon lotor*; Procyonidae) and indigenous canids, although spillings over into North American terrestrial mammals are likely to have been relatively rare in the recent past (Mackenzie *et al.*, 2016).

A survey of the incidence of reports of rabies in skunks and raccoons over the period 1992-2011 suggested that spill-overs were more likely from the latter into the former ; Interpretation of the data is confounded by the general lack of genotyping of the rabies variant involved (thus reports for skunks included areas where either the dog- or the bat-derived variant has been

identified); and thus whether, rather than each 'spill-over' being a one-off dead-end, the virus might have established at least 'sputtering' infections in the new host species. Nevertheless, Wallace *et al.* suggested various explanations for the evident bias towards raccoon-skunk transmission: apart from possible genomic preadaptations (or the lack thereof) on the part of the viruses involved, other likely influences included ecological and behavioural factors.

## 3. Conclusions

Available evidence suggests that most members of the genus *Lyssavirus* are relatively restricted in their taxonomic and geographic distribution. The major exception is *Rabies virus* which is enzootic in two different mammalian orders; lesser ones are the Australian bat virus (Marston *et al.*, 2018) and European bat lyssavirus type 1 (Serra-Cobo *et al*., 2002, 2013; Amengual *et al*., 2007; López-Roig *et al*., 2014), which infect more than one family of bats: in the case of the latter, this allows the virus to spread and be sustained in locales where the presumed normal host genus is absent.

Thus the rabies virus is extensively distributed in the Old World. Whilst it has established infections in a variety of wild species, the main reservoir host would appear to be dogs.[22] For rabies to persist in dogs, the population densities of the latter need to exceed a critical community size and the associated threshold for establishment ($S_T$: Dobson and Carper, 1996; Begon, 2009). Important in this regard is Bingham's (2005) distinction between maintenance infections (those which last for varying periods within a circumscribed population before dying out) and persistent ones (where interactions between different host populations can lead to a sustainable self-perpetuating cycling of the virus amongst them). In contrast to the discrete family-based units (packs)[23] characteristic of wolves (from which they were domesticated), present-day dogs are more individualistic and more likely to free-range and interact as a metapopulation (Marshall-Pescini *et al.*, 2017). Thus, whereas any infection in wolves is presumably most likely to be contained within the pack itself (given that these social groupings each range within and defend their own territories), the more dispersed interactions amongst dogs (especially those which have run wild, including so-called 'superspreaders' as a result of

[22] Marston *et al.* (2018) have pointed out that 'reservoir' in the case of dog rabies differs from the typical situation where a virus is nonpathogenic, or at least causes 'minimal disease', in its natural host; they suggest that this reflects a recent spilling over where the virus has evolved increased virulence to take advantage of the increased potential for transmission in terrestrial mammals.

[23] Comprising a breeding pair and their offspring; in present-day populations, the latter normally disperse and inter-mix when they become sexually mature at two-three years old, to establish their own family-units: http://westernwildlife.org/gray-wolf-outreach-project/biology-behavior-4/; http://www.wolf.org/wolf-info/basic-wolf-info/biology-and-behavior/.

the behavioural effects of infection: Brunker *et al.*, 2012) serve to increase the effective population size and thus the potential for rabies and other infections to become enzootic if host densities are sufficiently high (see also Sections III.6 and IV.3.iv). Hence rabies today may be a product of an early impact of humans on their environment through the domestication of dogs; the spread of the Cosmopolitan strain would be the most recent manifestation of this. Thus there has also been a similar recent spread of rabies virus onto the islands of Southeast Asia, involving regional strains rather than the Cosmopolitan clade, as a result of human-mediated movements overcoming physical dispersal barriers (reviewed by Munro, 2017; see also Brunker *et al.*, 2012).[24]

As noted above, it is generally assumed that lyssaviruses originated in bats. Based on known sequences, *Rabies virus* might have arisen as a result of a spill-over from fruit-bats in the Indian subcontinent or more eastern regions. Thereafter, infection presumably spread outward with different geographic biotypes emerging in areas with sufficiently large dog populations, and the Cosmopolitan strain becoming the most widespread. It was originally thought that the indigenous American clade, with its different biotypes primarily in New World bats, originated as a spill-over of the Cosmopolitan strain (presumably into vampire bats) from dogs brought over by European colonialists, but this is now considered unlikely based on historical and phylogenomic evidence. Arising out of this, one possibility is that the virus was introduced with dogs during the early spread of humans from northeastern Asia, given genomic evidence that domesticated dogs accompanied these early movements (Leonard *et al.*, 2002; van Asch *et al.*, 2013; Grimm, 2015; Frantz *et al.*, 2016; MacHugh *et al.*, 2017). However the small numbers of dogs in the founder population(s), the succumbing of those infected with rabies during the initial settlement and traversing of Beringia and/or their subsequent dispersal across the continent may have meant that any pre-existing infection was not sustainable. If it was sustainable at least initially, this might have led to spill-overs into one or more native species (such as seen today with the strain introduced by European colonialists), including vampire bats (which had a more extensive northerly distribution than today: Fenton, 1992; Naish, 2013), to then spread to other species of bat. This is one of the scenarios considered further in Section V.2.

[24] Similarly, for the recent indigenous (presumably bat-derived) American variant circulating in raccoons, humans have been responsible for its spread north from its origin in Florida (Brunker *et al.*, 2012).

Alternatively, the rabies virus may have been introduced to the Americas by movements of bats themselves. On the one hand, assuming an ancient origin, Rupprecht *et al.* (2017) have suggested that the indigenous American clade spread more than 65 million years ago with bats[25] emigrating from Africa to South America across the widening Atlantic Ocean after the splitting up of Gondwanaland in the late Cretaceous. Alternatively, the movement could have been from northern Asia (the apparent 'epicentre' for all lyssaviruses: Hayman *et al.*, 2016) across Beringia and/or the Bering Strait when environmental conditions were clement,[26] without the tight time constraints of an 'out of Africa' hypothesis:[27] that such long-distance movements would be feasible is indicated by the fact that, other than a species of seal, the only other mammal native to Hawai'i is the Hawaiian hoary bat (*Lasiurus cinereus semotus*), a subspecies of a vespertilionid bat widespread in the Americas, with evidence for at least two trans-oceanic influxes leading to founder populations (Bonaccorso and McGuire, 2013; Russell *et al.*, 2015). Related to these scenarios, Arita *et al.* (2014) concluded that two families of bats (the Emballonuridae and Molossidae) colonised South America from Africa,[28] whereas a third, the vespertilionids, spread into North America from Eurasia; thereafter, the joining of the two Americas at the Isthmus of Panama about five million years ago led to the Great American Biotic Interchange of bats as of other species (although some species of bats had crossed the dwindling space between the two continents for a considerable period prior to this: Arita *et al.*, 2014). Amongst the Vespertilionidae, genomic analyses suggest the common ancestor of present-day *Myotis* spp.[29] originated about 21 million years ago in the early Miocene (a relatively cool period) in the Oriental region, to thereafter spread outward and diversify into ten clades (Ruedi, *et al.*, 2013). Of these, clade I is found almost exclusively in the Americas, with the exception of the *My. brandtii* lineage from the Palaeoarctic, which lies embedded genome-wise between the two New World subclades (Stadelmann *et al.*, 2007; Ruedi *et al.*, 2013; Platt *et al.*, 2017).[30] Thus if rabies was introduced to the Americas at or after that time,

[25] Rupprecht et al. (2017) speculated that it may have 'spilled over' *via Eptesicus* or *Myotis* spp. (*cf.* Arita *et al.*, 2014; and see below).
[26] This connection has occurred intermittently during the past 63 million years (Eick *et al.* 2005).
[27] Present-day bats are found in Alaska south of the Arctic Circle (Parker *et al.*, 1997).
[28] Although it is generally considered that the Phyllostomidae (including vampire bats) arose in South America, there is some reason to doubt this (Arita *et al.*, 2014).
[29] This genus is characterised by having members which have evolved at relatively high latitudes (Ruedi *et al.*, 2013), and include the most northerly populations of bats in Alaska (Parker *et al.*, 1997).
[30] *cf.* Marston *et al.* (2018) who concluded, rather mysteriously, that "although *Myotis* species from America and Europe are morphologically similar, phylogenetically they are unrelated," and thus ruled out this hypothesis.

this may have been through these bats, to thereafter diversify with the radiating bat populations there.

Regarding the promiscuity of the rabies virus in relation to the species which it can infect and, in some cases, establish sustainable circulating biotypes over a period at least, Troupin *et al.* (2016) concluded that there is no evidence for any genetic predisposition to being able to successfully spill over into a particular new host species and thereafter evolve as an enzootic strain in this alternative reservoir. Instead, such events would appear to have occurred by chance, spread over time and space associated with propitious ecological factors, including adequate population sizes and densities.[31] Thus Wandeler (2004) noted that the biotype of rabies which emerged in red foxes in Europe in the middle of the last century has been largely confined to these and to raccoon-dogs, and has not spilled over to establish sustained infections in other wild carnivorans or in domestic dogs and cats. Because stone martens (*Martes foina*) and cats, at least, were considered likely to have local population densities equal to or exceeding those of red foxes, it was suggested that other important factors might be due to reduced susceptibility and/or less profuse salivary excretion; moreover both these factors may favour the acquisition of herd immunity due to the resulting abortive infections. In addition, as noted above with regard to skunks and raccoons, behavioural and ecological factors likely play a role (Wallace *et al.*, 2014).

In bats, particular strains of the rabies virus are generally restricted to their own narrow reservoir, typically to one host species, with any spill-overs being favoured between closely related species (Streicker *et al.*, 2010; Mollentze *et al.*, 2014). Some genomic analyses suggest that convergent changes in the amino acid sequences of a number of the proteins in the bat virus may have predisposed variants to become infective in their potential new host species (Kuzmin *et al.*, 2012). More generally, Mollentze *et al.* (2014) argued that the evident limited success of establishing spill-overs in terrestrial mammals as well as bats is the result of a dynamic interplay between (i) overt ecological factors with regard to the transmission between reservoir hosts and potential spill-over species; and (ii) intrinsic 'physiological' factors which are likely to be constrained by how closely related the original and possible new host species are (their 'phylogenetic distance'). Moreover, if subsequently transmitted within the new host species, the infection dynamics are likely to be 'sputtering' or only transiently enzootic

[31] Marston *et al.* (2018) have argued that pre-adaptation occurred with the original evolution of the rabies virus in bats, such that subsequent adaptive changes have been mere 'fine-tuning' of the virus after it has spilled over into a potential new host species.

(respectively Wolfe *et al.*'s [2007] stages 3 and 4; see also Woolhouse *et al.*, 2012) before dying out; a potential exception would be the emergence and spread of a strain of rabies in European red foxes, although this has since been limited by pro-active containment measures (Wandeler, 2004).

Thus, whilst Holmes *et al.* (2002) found limited evidence for positive selection across the then-available *Rabies virus* sequences as a whole, Mollentze *et al.* (2014) noted that a survey which focused on the indigenous American clade in bats (Streicker *et al.*, 2012) found evidence for periodic occurrence of such events associated with host-shifts: hence an all-encompassing analysis ignores more 'local' genomic changes as background noise, rather than evidence suggesting adaptation to a particular host species (but see Rupprecht *et al.*, 2017). Also, for the Cosmopolitan clade in North America, the use of so-called deep sequencing to identify the various variants of a rabies virus in particular cases of infection associated with a spill-over from a striped skunk reservoir into grey foxes indicated that the dramatic increase in the occurrence of outbreaks in the latter in 2009 was as a result of the selection for rare variants which were already evident in both skunks and a northern population of foxes in 1995 (Borucki *et al.*, 2013). Thus it was argued (Mollentze *et al.*, 2014; see also Marston *et al.*, 2018) that productive spill-overs might be the result of 'pre-adaptive' variants in the original reservoir which could, given the appropriate ecological conditions, spread and thereafter evolve in the prospective new host species; they further argued that experimental studies involving other species or cell-lines derived from these supported the capacity for such adaptive evolution.

On the other hand, phylogenomic analyses indicate that the genetic diversity of a host population is a good predictor of that of its associated viral subpopulation (presumably reflecting both sides of an ongoing arms-race), with evidence for increased host genetic diversity in reservoir hosts being correlated with greater evidence for positive selection on the part of their viruses, the sites in the latter varying with the reservoir species (Rodríguez-Nevado *et al.*, 2018). Furthermore, there was some suggestion that the associated viral diversity of certain reservoir species may predispose the latter to serve as sources of spill-overs (typically dead-end, in more ways than one) to other mammalian hosts, presumably because of the greater chance of potentially optimal variants pre-adapted to the new environment; however, again, ecological and other factors are likely to play a moderating role (Rodríguez-Nevado *et al.*, 2018).

The foregoing has given a brief review of the known diversity of lyssaviruses and broad aspects of their proposed evolution, and has referred in passing to some aspects of their infective

biology. The following section will consider the last in more detail for terrestrial mammals, mainly with regard to *Rabies virus*.

## III. Rabies-Related Virus Infections in Terrestrial Mammals

The following will briefly describe some of the key points regarding the aetiology and consequences of infection with *Rabies virus* (mainly based on observations on dogs and humans), followed by an overview of selected *in vitro* and *in vivo* studies with this and other lyssaviruses which have given insights into these. Thereafter, cases where a typical infection fails to become established in terrestrial mammals will be considered.

### 1. The Establishment of Infection

The standard scenario is that this occurs when the bite of an infected animal[32] inoculates virus-laden saliva into a potential future host; successful transmission requires infection to spread to neurones in the central nervous system, this being more effective with deeper bites in the case of dog rabies (reviewed by Hemachudha *et al.*, 2002, 2013; Jackson, 2013). There is evidence that both motoneurones innervating the inoculated muscle and the latter's associated sensory proprioceptors may be targeted by the virus, although the former may be more susceptible in the short term (Lafon, 2005; Dietzschold *et al.*, 2008); alternatively, at least for so-called CVS strains (developed in mice; see Section III.3.i), sensory neurones may instead be infected indirectly in primates, rats, and guinea pigs, as a result of trans-neuronal spread from infected motoneurones through local (presumably feed-forward) circuits within the central nervous system (Ugolini, 2011; Hemachudha *et al.*, 2013). Whichever route pertains, dog rabies may lead to infection of sensory neurones, associated with neuropathic pain, pruritic itching and other symptoms at the previously-bitten site during the subsequent prodromal phase (see below); this is more frequent in cases with bat-derived viruses (Hemachudha *et al.*, 2002, 2013), reflecting a potentially more diverse set of target tissues where even superficial bites may be infective (see Section III.3.ii).

The viral envelope's glycoprotein is initially responsible for interacting with potential future host-cells through 'receptors' (henceforth identified as acceptors: Munro, 2017)[33] on the

[32] Whether in the profromal phase or subsequently when clinical infection becomes manifest.

[33] Whereas a receptor is a macromolecule produced through natural selection in order to detect a particular 'event' of relevance to the organism involved (*e.g.* acetylcholine by the nicotinic receptor), this is not the case for the molecules coopted by viruses: indeed, natural selection may operate on potential hosts to favour the production of variants of the 'receptors' (*i.e.* acceptors) with which virions cannot interact in order to gain entry to a cell.

surface of the latter in order to gain entry to their target. The nicotinic acetylcholine receptor on somatic muscles has been identified as one such acceptor: it has been proposed that an affinity for the nicotinic receptor may serve as a means for concentrating the virus in the synaptic cleft to enhance neuronal uptake; and/or be associated with an initial 'booster' of the original inoculum through productive infection of the muscle cells to the same end (Dietzschold *et al*., 2008; Schnell *et al.*, 2010; Ugolini, 2011; Scott and Nel, 2016).[34] According to the latter hypothesis, the variability in latency before infection becomes manifest relates to the amount of time required for the virus to propagate in the muscle to a level where it can then successfully infect the associated neurones. Nicotinic receptors are not present on nerve terminals at the neuromuscular junction, meaning that other acceptor molecules must be involved for uptake by motoneurones. From experimental studies, one prime (but not essential) candidate is the neural cell adhesion molecule (N-CAM) (Lafon, 2005; Jackson, 2007; Ugolini, 2011); this is reinforced by the fact that it is widespread on the presynaptic terminals of neurones (being important in neuroplasticity: Rønn *et al*., 1998), including components of the autonomic nervous system (Faure *et al.*, 2007), as well as on muscles and the Schwann cells insulating motoneurones (Covault and Sanes, 1986). However other (co-)acceptors may also be involved, with different ones possibly being implicated in sensory cells compared with motoneurones (Lafon, 2005; Schnell *et al.*, 2010).

Having entered target neurones after a latent period of variable duration (Perhaps related to the initial replication in the inoculated muscles), the virus' glycoprotein then hijacks the fast retrograde axonal transport system in order to be carried back (3 mm/hr: Dietzschold *et al*., 2008) to the infected neurones' somata in the central nervous system.

## 2. The 'Normal' Progression of Infection

Having gained access to the central nervous system, the virus is now unleashed to multiply and spread retrogradely along neural networks to progressively higher-order neurones (Ugolini, 2011; Hemachudha *et al.*, 2013). Thus, having replicated within the soma of a neurone, virions are released from the dendrites to be taken up by presynaptic terminals by way of N-CAM or other acceptor mechanisms (Lafon, 2005, 2016; Jackson, 2007; Ugolini, 2011). Whilst a component of the viral capsid has been identified as promoting fusion between adjoining cells for the onward spread of the virus infection (Dietzschold *et al*., 2008), this may be a derived characteristic of certain *in vitro* strains (Ugolini, 2011), reinforcing concerns that cell lines may

[34] Laboratory CVS strains are unable to infect muscle cells: Ugolini (2011).

behave atypically *ex vivo* (Wunner, 2007). In contrast to the effects of acute infections of the CNS by other viruses (Griffin, 2003), there is little evidence for neuro-inflammatory pathology; apart from strategies to subvert the host's immune responses,[35,36] the main effects are to reduce the activity of house-keeping genes and the production of proteins as a result (Lafon, 2005; Dietzschold *et al.*, 2008; Warrell, 2009; Hemachudha *et al.*, 2013; see also Section V.1).

Thus the rabies virus is optimally adapted to seek to minimise short-term damage to its current host, in order to achieve the necessary end-result for possible onward transmission: the spread of contagion *via* the autonomic nervous system to the salivary glands, leading to the infection of the latter and the production of a virion-rich saliva as a vehicle to this end in carnivorans (Jackson, 2008, 2014).[37] Other tissues are also infected as a result of this centrifugal spread, including skeletal and cardiac muscle, the kidney and adrenal glands, the pancreas and nerves around hair follicles (Warrell and Warrell, 2004; Jackson, 2007; Warrell, 2009). This switch to anterograde spread of virus within the autonomic nervous system, for example, is proposed to be the result of passive diffusional processes due to the progressive overwhelming of neuronal function in the central nervous system at large (Ugolini, 2011). Hemachudha *et al.* (2013) considered that the most likely route for centrifugal spread in humans was through sensory afferents, as well as *via* lymph nodes, thereby possibly serving to trigger activation of the immune system and thus potentially expedite the demise of the current host.

As a result, after a variable latency (typically up to 3 months; but see below), disease first becomes evident during a prodromal phase lasting 2-10 days which is characterised by nonspecific symptoms such as general malaise, together with abnormal sensations associated with the site of original infection (Hemachudha *et al.*, 2002, 2005; Jackson, 2007; Constantine,

[35] Whilst the initial infection is potentially exposed to the immune system at large, there is generally no evidence for an acquired immune response prior to the prodromal phase (Johnson *et al.*, 2010; Scott and Nel, 2016).

[36] One additional intriguing possibility – which would reduce the need to synthesise glycoproteins and thus the risk of provoking an early immune response (Lafon, 2008, 2011) – is that viral RNA might itself be transferred retrogradely, at least initially, *e.g.* by taking advantage of the *Arc* gene. The latter has been independently co-opted in insect and tetrapod vertebrate lineages from ancient retroviral infections, with the resulting gypsy retrotransposons being translated and their proteins assembled into 'capsids' which act as packages to transmit *Arc* mRNA itself (and potentially other RNAs) across the synaptic cleft as a 'novel' means of inter-neuronal signalling implicated in learning and memory processes, although whether this is uni- or bidirectional has yet to be identified in mammalian models (Parrish and Tomonaga, 2018). Presumably where this has occurred at least once amongst tetrapods and independently in *Drosophila*, other analogous such acquisitions will be found, as in the case of the also retrotransposon-derived syncytins important in the development of the placenta.

[37] It is likely that the infection of the salivary glands is dependent on the overall varicose innervation rather than through local spread of virus after focal infection (Charlton *et al.*, 1983).

2009; Warrell, 2009; Lafon, 2016). By this stage, the virus has become widely spread within the central nervous system, and has also spread to the salivary glands as a source of potential onward transmission (Hemachudha *et al*., 2002, 2013). Thereafter, the disease may proceed directly to a paralytic phase, so-called 'dumb' rabies,[38] with passivity, muscle weakness and the resulting flaccid paralysis, the victim sinking thereafter into a terminal coma. The development of such symptoms during this phase in humans is very similar to that of Guillain-Barré syndrome and related autoimmune diseases,[39] typically non-fatal conditions where the immune system attacks the peripheral nerves seemingly in response to an infection although the syndrome itself is not contagious. This is more extreme in paralytic rabies, with evidence for inflammatory responses at the spinal level and degeneration of peripheral axonal myelin sheaths, with death being delayed in immuno-compromised cases (Hemachudha *et al.*, 2005, 2013). Thus paralytic cases have often been misdiagnosed, as suggested above for human infections from vampire and other bats before it was realised that they represented an additional spill-over source of rabies viruses (Warrell, 2009).

However this terminal stage is preceded in many infected terrestrial mammals[40] by a manifestation of the classical 'furious' (also called 'encephalitic') form of the disease, characterised by periods of arousal and hyperexcitability (including hallucinations and fear-induced agonistic behaviours)[41] interspersed with those of lucidity; this presumably reflects spread of infection to include the limbic system of the forebrain (Hemachudha *et al.*, 2005, 2013; Jackson, 2007; Warrell, 2009; Hanlon *et al*., 2013). Nonetheless, this manifestation of infection has often been misdiagnosed (Warrell, 2009). Its average latency to develop is comparable with that for the 'dumb' form, but it is of variable duration; thereafter, it may proceed to the latter, or else the victim may die as a result of cardio-respiratory arrest during spasms associated with feelings of terror but no feeling of pain: death typically occurs in half the time seen with paralytic cases. However the resulting behavioural changes serve to maximise the chances of onward viral transmission through biting of potential new hosts. There

[38] Humans and other animals are not necessarily rendered mute (Warrell, 2009).

[39] https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Guillain-Barr%C3%A9-Syndrome-Fact-Sheet

[40] About two-thirds of patients infected by dog-derived viruses; a similar proportion is seen in infections from bats in North America (*cf.* Section II.2.ii), although the clinical presentation differs, including more frequent local sensory effects (Udow *et al.*, 2013); for other animals, the result is typically furious in cats, mustelids, and viverrids and horses, but paralytic in foxes and bovines (Warrell, 2009).

[41] Often with the classic hydrophobic response in humans (not seen in other animals), where the desire to drink (*e.g.* to replenish losses from fever-induced sweating) is antagonised by efforts to swallow and the resulting pain mediated by autonomic effects (Warrell, 2009).

is evidence that the furious form of rabies in dogs is associated with earlier, more intense neuro-invasion (associated with faster intra- and thus intercellular transport) and less extensive inflammation than is the case with the paralytic form (Laothamatas *et al*., 2008; Shuangshoti *et al.*, 2016). However whether an infected individual first develops furious rabies or proceeds directly to the paralytic form (or develops a non-classic form of the infection) is independent of the strain of virus received from dog bites, presuming that there have been no post-infection mutations of the original strain (Hemachudha *et al*., 2002, 2013).

Some infections may only develop after a long latency. For example, Jackson (2007) reviewed three cases of immigrants to the United States – from Laos, the Philippines and Mexico – who developed rabies from a strain of virus which was characteristic of their countries of origin, indicating that they must have been infected, respectively, at least 11 months, four or six years previously; also, one Vietnamese immigrant in Australia in 1990 was likely to have been infected at least five years earlier. The delay presumably reflects prolonged quiescent infection at the site of inoculation prior to neuronal uptake, perhaps related to the dose of the original inoculum (Scott and Nel, 2016; see above). Other studies on humans have failed to find any relation between bite-location and the subsequent latent period, apart from evidence that this may be shorter for bites to the face (Warrell and Warrell, 2004). This contrasts with evidence that the likelihood of active infection is highest (50-80%) for bites to the head in humans, intermediate (15-40%) for those to the forelimbs and lowest (3-10%) in the case of leg bites; the underlying factors are unclear, although possibilities include the density of virus acceptors in the inoculated tissues and the degree of innervation of the latter, together with the amount of virus inoculated and the properties of the variant involved (Jackson, 2007). Associated with this is a progressive distance-related increase in the average latency for infection to become manifest (Warrell, 2009), superimposed on the initial (common?)[42] incubation time in the originally infected muscle.[43] Another variable is likely to be age and the relative development of the immune system, with young and ageing animals being more susceptible (Scott and Nel, 2016).

[42] Assuming comparable densities of nicotinic recepors on different muscles (Baer *et al.*, 1990).
[43] Given a transport rate of 3 mm/hr from the periphery (see above) translates to about 21 cm/month.

### 3. *In Vitro* Studies

These have proven insightful in studying some aspects of the basic biology of mainly the rabies virus. Whilst short-term studies have used explants of neural tissue, longer-term ones have used cultures based on neuroblastoma, kidney, fibroblast and epithelial cell-lines.

The following will first consider studies of cultures of the rabies viruses derived ('fixed') from those circulating in dogs; and then the apparently special case of one strain, the silver-haired bat rabies virus (SHBRV).

***i. Dog-Derived Strains*** The establishment of *in vitro* cultures of so-called 'street strains' of the virus isolated from rabid dogs using various cell-systems has led to the emergence and incidental selection of what are referred to as 'fixed' strains, such as variants of the challenge virus standard (CVS) and those used to produce vaccines; these have adapted to tissue-culture, emerging and thus being selected for *de facto* because they replicate the fastest with the least detrimental effects on the chosen host cell-type during successive passages *in vitro* (Dietzschold *et al.*, 1985, 2008). Thus genomic analyses of the glycoprotein and nucleoprotein genes of fixed laboratory strains indicate that purifying selection was a predominant force (possibility reflecting adaptation to infect the particular cell-type being used), in contrast to the limited evidence in favour of positive, potentially adaptive selection in the original wild strains (Holmes *et al.*, 2002). As a result, various so-called 'carrier' strains may emerge which differ in their pathogenicity when subsequently tested *in vivo* (see Section III.4).[44] An additional, perhaps related issue is that *in vitro* infections may lead to the production of defective-interfering virions, which have shortened strands of RNA as a result of deletions and which replicate at the expense of the original strain and its viable variants; the generation of these would seem to be selected against *in vivo*, perhaps because of their loss of anti-inflammatory activity (Wunner, 2007).

Thus, for example, Morimoto *et al*. (1998) found that passage of a fixed mouse-adapted rabies virus strain in hamster kidney (BHK) cells resulted in the rapid selection of a variant that differed genotypically from the dominant variant of the original virus isolated from mouse brain or after passage in neuroblastoma cells; this was associated with a phenotypic switch in the preference for particular cell-types, with the same variant consistently emerging during repeated transfection experiments into BHK cells. They concluded that this variant may be

[44] It would not seem to be known whether there has been a parallel co-selection for particular cell lineages *in vitro*, although this might seem unlikely with multiple passages.

present as a sub-population within the original mouse-adapted strain, reflecting the 'quasispecies-like' diversity expected as a result of the error-prone nature of replication in RNA viruses;[45] more generally, they proposed that this may be important in allowing the virus to cross species-specific and other barriers. In this context, it is pertinent to note that Wunner (2007) argued that the presence of such (quasi-) quasispecies may be a by-product of the artificial conditions of culturing *in vitro*, due to the cells behaving differently from how they do *in vivo*.

A number of studies have been identified mutations of amino acids 330 and 333 in the antigenic site III of the glycoprotein as important in pathogenicity in fixed rabies strains; these are normally basic but the virus is unable to enter cultured motoneurones when both are substituted (Coulon *et al.*, 1998; reviewed by Jackson, 2007; Dietzschold *et al.*, 2008). On the other hand, the double mutant retained the ability to infect BHK cells, neuroblastoma cells, and freshly prepared embryonic motoneurones, but with a lower efficiency than for the reference CVS strain used; however, continued culture of embryonic motoneurones led to their becoming resistant to infection by the double mutant whilst still being susceptible to CVS infection. It was proposed that this reflected a cardinal role for two different types of acceptor in mediating infection with rabies virus; and that one of these, on differentiated neurones, is not recognised by the double mutant (Coulon *et al.*, 1998). Other amino acids in the same region of the glycoprotein also play a role in the virus' pathogenicity *in vitro* (Takayama-Ito *et al*., 2006a).

***ii. The Case of the Silver-Haired Bat Rabies Virus (SHBRV)*** Comparative experimental studies of a variant of the Cosmopolitan strain of the rabies virus isolated from a wild Texan coyote (*C. latrans*) with that from the brain of a human in California infected from the silver-haired bat (*Lasionycteris noctivagans*)[46] identified important differences between the two, presumably related to differences in the amino acid sequence of the glycoprotein; each strain was a stock prepared from a single passage of the primary isolates in suckling mouse brains to minimise fixation problems as a result of artificial selection for culture conditions. Whilst both strains were equally efficacious *in vivo* when injected intracranially or intradermally into mice, the bat-based isolate was less neuro-invasive than that from the coyote when administered

[45] See Holmes (2010) for a critique of the application of the quasispecies concept in virology.

[46] This and another small solitary tree-roosting species, the tri-coloured bat (*Perimyotis* [= *Pipistrellus*] *subflavus*), account for about two-thirds of human spill-over cases in the USA, despite not normally living near houses; the two species have closely-related rabies virus variants in a different clade from those of other bats (Messenger *et al*., 2003; Davis *et al.*, 2006; Kuzmin *et al.*, 2011; Kuzmina *et al.*, 2013).

intranasally or intramuscularly (Morimoto *et al*., 1996). *In vitro* studies demonstrated that, whilst both isolates were equally effective in infecting a mouse neuroblastoma cell-line, the infectivity of the bat isolate was much higher than that of the coyote in a fibroblast and an epithelial cell-line. This, together with differences between the two isolates in cultures kept at 34° C (*cf*. the normal 37° C) – fuseogenic activity and cell-to-cell spread of SHBRV was limited to the lower temperature – led Morimoto *et al*. (1996) to propose that the adaptations of this bat isolate may mean that the initial infection of epidermal cells (which are at a lower temperature) by even a small number of virions in a superficial scratch or bite may lead to the amplification of the virus to thenceforth spread to the central nervous system. Thus the authors concluded that the adaptations of the virus from this bat may help to establish and amplify an initial infection of epidermal cells from the small inoculum likely to have been received from a nip or scratch, as a prelude to invading nerve fibres in order to spread centripetally into the central nervous system: this will be considered further in Section V.2.

### 4. *In Vivo* Studies

Although mice and rats are standard laboratory models for a diversity of areas for biological study, rodents are not generally considered to be natural reservoirs for rabies: the argument is that, being short-lived and likely to be killed by rabies-infected predators anyway, the small species at least are dead-end hosts (Hanlon *et al.*, 2013). An exception would seem to be China, where there is evidence for a rabies virus in rats and other local rodents which is different from that circulating in dogs, with evidence for human spill-overs in the 1990s (reviewed by Wang *et al.*, 2014).

When the rabies isolate from a naturally-infected European fox was serially-passaged by the primary infection of various other host mammals or cell-lines with subsequent trans-generational inoculations within the particular experimental regime being used, it was found that mutation rates were higher when the subjects were mice *in vivo* than for dogs and cats and for *in vitro* studies (Kissi *et al.*, 1999). Changes (mainly nonsynonymous: implying positive selection) accumulated over passages, being the more so for the glycoprotein gene than for other components of the virus.

Nevertheless, mice are routine subjects for *in vivo* tests of other potentially rabies-infected animals, typically through direct intra-cranial injections. However this has its experimental limitations in the study of a real-world *in vivo* situation where any infection is likely to originate peripherally; the continued use of intracranial injection serves as a base-line to compare the

effects of other modes of inoculation. Thus up to three other modes of administration have typically been used for experimental studies. The most frequently used additional protocol is *via* intramuscular inoculation, but other studies have also used intradermal injections and intranasal administration; whilst some have also considered intravascular inoculation. Comparisons amongst studies are often confounded not only by differences in the particular strains of the rabies and other lyssaviruses and of the recipient test-animals used but also in methodological details of how the virus is administered. Thus, for nasal infections of the brain in mice, Rosseels *et al*. (2011) found that effectiveness depended on inoculum volumes and the nature of the anaesthetic used.

Apart from the occurrence of deaths over a predetermined period, other parameters monitored in these and the survivors have generally included the presence of virus in oro-pharyngeal swabs during the course of the experiment, together with serum antibody levels (typically in a neutralisation assay: Moore and Hanlon, 2010), along with the detection of virus in the brain and salivary glands and possibly various other tissues *post mortem* or at the end of the experiment. Again, methodological differences often make comparisons between studies difficult, particularly where lyssaviruses other than the rabies virus are used, due to the use of different strains by different laboratories as well as differences in the detection methods.

Most information is available for different strains of the rabies virus isolated from dogs. Tests with 'wild' rabies viruses have often provided inconsistent results. Thus Banyard *et al*. (2014b) found variability amongst mice subjected to multiple foot-pad inoculations of rabies virus or European bat lyssaviruses types 1 or 2. Whilst about a quarter died, including some of those receiving the lowest dose (equivalent to about four virions/inoculum), the pattern of mortality and/or the serological response was unpredictable. The variability in sero-conversion was suggested to reflect that in cell-mediated clearance by the innate immune system, at least in part.

Consistent with *in vitro* studies, fixed rabies strains with mutations of both amino acids 330 and 333 in the glycoprotein to produce non-basic substitutions are non-neurotropic *in vivo* and so are apathogenic in adult mice when injected intra-cranially or -muscularly (Coulon *et al.*, 1998; see also Takayama-Ito *et al*., 2006b). When tested *in vivo*, other fixed strains typically have a reduced neuro-invasiveness compared with the street strains whence they originated, associated with a faster rate of replication and their induction of stronger, more rapid immune responses *in vivo*, more especially in adults with associated apoptosis (Scott and Nel, 2016). Thus, for example, the attenuated strain CVS-F3 led to only a transient infection of adult mice

after nasal inoculation, associated with a strong inflammatory response in the brain as well as the production of neutralising antibodies (Hooper *et al*., 1998); further experiments indicated that the infection was contained as a result of the conjoint action of the innate and acquired immune systems to mediate, respectively, the inflammatory response and B cell-mediated antibody production. Other studies with an attenuated rabies virus strain (HEP-Flury) administered by the same route found only transient, subtle clinical signs, in contrast to the virulent CVS-11 strain; this was associated with the earlier activation of an innate immune response, together with an increased acquired immune response and the more rapid clearance of virus from the brain, as compared with the later, more extensive neuro-inflammatory response with CVS-11 (Zhang *et al*., 2016). Similarly, intracerebral injection with an attenuated CVS strain from passage in BHK cells led to an early inflammatory response in mice, in contrast to that with SHBRV, indicating that the latter is also able to evade the host's innate immune system (Wang *et al*., 2005).

On the other hand, experiments on mice with a rabies isolate from the bat *Ep. fuscus* found that the highest rate of mortality was after intranasal inoculation, presumably as a result of direct access to the brain through olfactory pathways;[47] intradermal and especially intramuscular administration instead led mainly to seroconversion (Ndaluka and Bowen, 2013). Exposure of mice to aerosols from three indigenous American bat variants of the rabies virus[48] resulted in the death of four of the nine with evidence for rabies infection, whilst the survivors developed neutralising antibodies (Davis *et al.*, 2007). Whilst the relevance of these findings to the situation 'in the wild' is unclear, it indicates the potential to evolve such a means for onward transmission given the appropriate environmental opportunities (see Section IV).

Not only is SHBRV potent when inoculated intramuscularly in mice but also when an identical dose was instead injected into the tail vein; this was not seen with a standard strain of rabies derived from dogs (Preuss *et al*., 2009). Infection was apparently mediated by uptake *via* neurones of the hypothalamo-hypophysial system[49] leading to a lethal encephalopathy.

[47] Note that this implies anterograde transport of virions, in contrast to the general view that this is normally retrograde during most stages of infection initiated elsewhere (see Section III.2).

[48] One isolate from each of *Ep. fuscus*, *T. b. mexicana* and the 'solitary' bat *L. noctivagans*' SHBRV.

[49] One of the so-called circumventricular organs, where there is no blood-brain barrier: these serve as additional means for exchange of information between the central nervous system and the body at large. By extension, other such CVOs also represent potential means for the virus to bypass the blood-brain barrier and enter the central nervous system.

Amongst other members of phylogroup I, McColl *et al.* (2007) reported that tests of the Australian bat lyssavirus over a three-month experiment resulted in transient clinical symptoms in three of five domestic dogs with subsequent seroconversion but no evidence of salivary viral RNA or antigen when necropsied three months later, although two of the dogs had neutralising antibodies in the cerebrospinal fluid; there was much lesser response with cats.

Somewhat similarly, intramuscular inoculation of *V. vulpes* with *European bat lyssavirus type 1* led to early rabies-like symptoms in all eight foxes from which they recovered but with subsequent relapses in all but one (Vos *et al.*, 2004a); whilst neutralising antibodies were detected in the serum and brain, no antigen was found in the brain, salivary gland or other tissues tested, including in the three animals which were sacrificed. On the other hand, there is evidence that strains of this virus from different bats and other sources differ in their pathogenicity in mice, despite only minor variations in sequence (mainly as a result of insertions or deletions): whilst all mice receiving intracranial injections succumbed, mortalities were fewer with intramuscular and intranasal inoculations (Eggerbauer *et al*., 2017). An earlier experiment found that, whilst mice were susceptible to intranasal inoculation with either a mouse-adapted standard (CVS) strain or European bat lyssavirus type 2, only the former was efficacious after inhalation of aerosolised virus (Johnson *et al*., 2006b).

Experiments with ferrets (*Mustela putorius furo*) and mice using European bat lyssavirus type 1 found that all receiving the higher intramuscular dose ($10^{6.0}$ FFU) died, whereas only a proportion of either species succumbed to a lower dose ($10^{4.0}$ FFU) within the first 18 days; other animals receiving a comparable dose of European bat lyssavirus type 2 survived apart from one of the five mice (Vos *et al.*, 2004b). Moreover, whilst all ferrets seroconverted (progressive in the case of some type 1 survivors *cf.* transient in some of those receiving type 2), virus could be detected in various tissues at the end of the three-month experiment, but not in saliva swabs during the intervening period. Subsequently, as noted above, Banyard *et al*. (2014b) found variability amongst mice subjected to multiple foot-pad inoculations of rabies virus or European bat lyssavirus types 1 or 2.

Badrane *et al.* (2001) found evidence that two members of phylogroup II (the Lagos bat virus and the apparently non-bat Mokola virus) were less pathogenic in mice than was the case for two from phylogroup I (the rabies virus and European bat lyssavirus type 1): whilst infective after intracerebral injection, they were ineffective after intramuscular inoculation. This was attributed in part to the presence of non-basic amino acids at positions 330 and 333 in the glycoprotein of these phylogroup II viruses. That they remained effective after intracranial

injection was suggested to be due to other mutations within the portion of the ectodomain responsible for interacting with neuronal acceptor-targets. On the other hand, a subsequent study using a greater number of isolates of the Lagos bat virus[50] tested intramuscularly found that some of these were as or more pathogenic than the strain of rabies virus used; it was concluded that other mutations also played a compensatory role (Markotter *et al.*, 2009).

## 5. Apparently Atypical Infections in Natural Populations of Terrestrial Mammals

Fekadu (1991; see also Cleaveland and Dye, 1995) has reviewed the nature of atypical infections where nonvaccinated dogs are asymptomatic at the time when they are sampled, despite evidence for likely past infections. One category of these is represented by cases where symptoms do not develop within the expected time-frame after an experimental inoculation or a likely natural one, with no evidence for viral shedding in the saliva. This comprises cases where there may be either (1) a prolonged latent period prior to the development of clinical symptoms, as noted above for humans (the 'long incubators' of Bingham, 2005); or (2) aborted (or 'inapparent') infection, associated with the development of an acquired immune response with neutralising antibodies in the serum and an anamnestic response to re-infection despite the absence of any clinical signs of disease or virus excretion.[51] Fekadu's other category constitutes animals which became ill but then recovered (possibly under-reported due to infected animals showing signs of illness typically being killed for post-mortem investigation), with the subsequent appearance of neutralising antibodies in the cerebrospinal fluid. This category included animals which were infectious carriers, with prolonged excretion of virions in the saliva by now healthy animals; these potentially represent a long-term pool to maintain viral circulation within the host reservoir.

In what would seem to be a case of poor reportage, Minard (2009) stated that a bat virus had spilled over into skunks and, latterly, foxes in northern Arizona and that "the strain appears to have mutated so that [they] are now able to pass the virus on to their kin – not just through biting and scratching but through simple socializing, as humans might spread a flu. … Skunks have already been proven to be passively transmitting the strain to each other, as documented in a 2006 study in the journal *Emerging Infectious Diseases.*" However, the presumed study cited (Leslie *et al.*, 2006) makes no mention of any such non-terminal, non-aggressive infection

[50] There is evidence for four different strains, with members of A and C being tested.

[51] Typically, antibodies are first detected in the blood at or shortly before classic symptoms of infection become manifest, and then in the cerebrospinal fluid at the peak of viral production (Johnson *et al.*, 2010).

in striped skunks (*M. mephitis*), never mind that it was spread by aerosols or other casual contact.

The following will consider reports regarding abortive/inapparent cases and the possibility of carriers, including humans and the special case of one study on spotted hyenas (*Crocuta crocuta*).

***i. Abortive/Inapparent Cases and the Possibility of Carriers*** Fekadu (1991; see also Warrell and Warrell, 2004; Jackson, 2007) has reviewed cases where apparently healthy individuals in various Carnivora were seropositive for the rabies virus, including those where viral sequences or active virions have been identified in the saliva. Fekadu *et al.* (1981 [abstract]) found that a dog inoculated with an isolate from an apparently healthy Ethiopian dog which excreted rabies virus[52] in its saliva over a period of more than nine months. A subsequent study reported another case where a female dog administered the same virus developed disease but then recovered when given "supportive treatment"; rabies was subsequently isolated from saliva collected 42, 169 and 305 days after recovery and, when she died giving birth to two stillborn puppies sixteen months after recovery, from the palatine tonsils[53] but not from the brain (Fekadu *et al.*, 1983 [abstract]). With regard to dogs elsewhere, claims for carriers in China are controversial (Zhang *et al*., 2008; Hu *et al*., 2009; Wu *et al*., 2009). Similarly reports regarding one case of what was identified as a chronically infected dog in India are open to question (Jackson, 2007). Intriguingly, anecdotal reports indicated that the dogs apparently responsible for fatal infections through biting a girl about seven years previously in Laos, and a boy about four years prior in the Philippines, survived for at least a month after their attacks (Smith *et al.*, 1991). On the other hand, Nel and Rupprecht (2007; see also Aghomo and Rupprecht, 1990; Warrell and Warrell, 2004) refer to cases of *oulou fato*, an asymptomatic variant of rabies in dogs identified throughout much of sub-Saharan Africa in the early twentieth century which was considered to be less transmissible to humans and may still be circulating today. Thus Aghomo and Rupprecht (1990) characterised a rabies-type virus in the saliva of healthy dogs in Nigeria which was antigenically distinct from the strain used to vaccinate dogs there but which was pathogenic after intracerebral injection into new-born mice.

[52] Or at least a phylogroup I virus: its genome was not sequenced.

[53] Note that these lymphoid tissues (together with the adenoids and other components of Waldeyer's tonsillar ring), responsible for initiating innate immune responses to the presence of potential pathogens in the naso-oropharyngeal cavity, have been found to serve as a reservoir for other viruses in other species of mammal, even in the face of continuing neutralising antibody response: the Epstein-Barr virus (a gammaherpesvirus) and certain adenoviruses, parvoviruses and polyomaviruses (reviewed by Munro, 2017; see also Garnett *et al.*, 2009).

Furthermore, Cleaveland and Dye (1995) found that a considerable proportion of domestic dogs in areas adjoining the Serengeti National Park were seropositive for rabies or a related virus. Genomic studies of rabid dogs in Nigeria indicate that a distinct variant, Africa 2, is circulating in rabid dogs there and elsewhere in sub-Saharan West Africa as a long-term enzootic (Nel and Rupprecht, 2007; Zhou *et al.*, 2013; Dzikwi *et al.*, 2017),[54] as well as members of the Africa 1 lineages of the Cosmopolitan strain (Hayman *et al*., 2011). More recent studies on apparently healthy dogs in Nigeria have identified rabies antigens in the brains (Aliyu *et al.*, 2010; Otolorin *et al.* 2014) as well as in the saliva (Mshelbwala *et al.*, 2013); it is not clear whether the dogs would have gone to manifest clinical symptoms.

Whilst cats are now recognised to be intermediaries in the spilling over of bat and Cosmopolitan lineages into humans with the elimination of dogs as a threat in North America, they are relatively resistant to the virus itself (Constantine, 2009). Thus there are reports of "remarkable" (Jackson, 2007) individual cases where there was evidence that a bat rabies virus may establish chronic or recrudescent infections (Perl *et al*., 1977 [abstract]; Murphy *et al*., 1980 [abstract]). Whether they might have represented potential carriers is open to question in the apparent absence of evidence regarding viral excretion in the saliva or through other routes, and whether they survived because of veterinary care.

Another feliform, the Indian mongoose (*Herpestes auropunctatus*), has been introduced to certain islands in the Caribbean based on the presumption that it would prove effective in the control of rodents and snakes in cane-fields; populations have been established which subsequently became a reservoir for various strains of the Cosmopolitan rabies virus circulating in the Americas as a result of spill-overs (Everard and Everard, 1992; Nadin-Davis *et al.*, 2008; Zieger *et al*., 2014). Studies on the small island of Grenada (≈ 310 $km^2$) over the period 1968-1972 found that the incidence of mongooses with rabies infections (based on a brain fluorescent-antibody test) varied between 0.5% and 3.7%; on the other hand, high rates of transmission within the population were evidenced by serum neutralising antibodies being found in 18.9% of the animals tested (Everard *et al.*, 1972, 1974). There was little evidence for transmission to domestic dogs (where there had been a vaccination drive) and cats or livestock as an expanded reservoir, so that spill-overs into humans were direct (see also Everard and Everard, 1992).[55] The authors found that more than half of road-kills had evidence for ongoing

[54] It is not clear whether the source of Zhou *et al.*'s virus was rabid or not.

[55] Vampire bats would appear to be absent (Everard *et al.*, 1972); whilst serological studies have identified prior infections of a rabies-type virus in indigenous bats (mainly the frugivorous *Artibeus*

brain infections; and that samples caught in baited traps "frequently found fluorescence in the brain of mongooses which apparently showed no abnormal … behaviour, although this was sometimes difficult to interpret in a small trap in strange surroundings. … The fact that these mongooses entered traps for food suggests that the disease had not progressed to the point of causing disorientation characteristic of attacking animals; nevertheless, it is presumed that they would eventually have died of rabies" (Everard *et al.*, 1974). Behavioural changes associated with overt evidence for rabies infection were mainly of the 'furious' kind (see also Everard and Everard, 1992). Subsequent studies (Everard *et al.*, 1981) found evidence for a progressive inverse relationship between the proportions of antibody-positive and rabies-positive mongooses over 1971-1974, with long-term survival of antibody-positive individuals over a period of up to 35 months. Everard and Everard (1992) noted that the mongoose population density, whilst fluctuating, is "almost certainly" higher than on other Caribbean islands whence it has been introduced; Everard and Everard (1988) contrasted the situation regarding seroprevalence with reports for red foxes where only up to 8% develop immunity (and typically much less: Wandeler, 2004). More recently, Zieger *et al*. (2014) found evidence that seropositive mongooses were initially restricted to northerly portions of the island to spread southwards over the period 2011-2014; this was associated with evidence for a recent bottleneck, suggested to be the result of recent governmental control efforts together with the impact of hurricanes in 2004 and 2005 on the indigenous mongoose population.

Unclear is the situation regarding the two subspecies of ferret badgers (*Melogale moschata*) from mainland China and Taiwan. One report for Taiwan, where the last reported case of dog rabies was in 1961, identified a rabies-related lineage in three sick animals submitted for pathological examination, and concluded from genomic analyses that there was a long-term "cryptic" circulation within the local ferret badger population prior to the evident outbreak in 2013 (Chiou *et al.*, 2014). Further surveillance of dead animals (including road-kills) indicated that there were geographically-distinct viral lineages on the island, consistent with the virus not being a recent introduction (Lan *et al.*, 2017; Shih *et al.*, 2017). On the mainland in southeastern China, Liu *et al.* (2010) identified two related viral strains in the local subspecies of ferret badger; there was a high seroprevalence amongst the live animals tested (69.6%; *cf.* 18.2% for dogs), and one of 56 brain samples was rabies-positive. The viral strain in the mainland population was different from that in other local fauna, including dogs where a new

*jamaicensis*), these cannot distinguish between the Cosmopolitan and indigenous American strains (Zieger *et al*., 2014).

clade is emerging (Wang *et al.*, 2014). Taken at face-value, the Taiwanese findings might suggest the recent emergence of a more virulent strain, but this presumes a constant, consistent background surveillance over a decade or more prior to the observed outbreak. Interestingly, Troupin *et al.* (2016) found that the evolutionary rates of the rabies strain in these, like that in African mongooses, was twice as fast as that for strains of dog rabies.

***ii. Possible Human Cases*** Whilst rabies is generally considered to be invariably fatal in humans, as with other animals there are reports of exceptions as evidenced by recovery from signs of illness and/or neutralising antibodies in the brain or cerebrospinal fluid (Fekadu, 1991). Some serological evidence suggests the possibility of aborted infections, recalling evidence that bites to the legs, in particular, are least likely to cause active infection for whatever reason (see Section III.2). Thus Jackson (2007) has reviewed evidence for low titres of rabies virus-neutralising antibodies having been found in seven of 20 Canadian Inuit hunters and two of 11 of their wives; and in five of 30 Florida raccoon hunters, but not in a control group of other hunters; and in 6.6% (15 of 226) of unimmunized students and faculty members of a veterinary medical school at the inception of a rabies vaccine trial. It was concluded that low-level exposure to rabies virus under natural conditions might have led to immunity, without the occurrence of clinical disease.

Associated with a recent outbreak of the furious form of the disease from a vampire bat strain of rabies in two isolated villages in the Peruvian Amazon, Lopez *et al.* (1992) found low levels of rabies-neutralising antibodies[56] in seven of the 48 healthy relatives of the victims (the only individuals tested), although the authors considered that this was a result of non-specific cross-reaction. Similarly, a more recent study by Gilbert *et al*. (2012) of villagers from the Peruvian Amazon found that rabies lyssavirus neutralising antibodies were detectable in seven of 63 samples (three with a titre > 0.5 IU/ml), all but one of whom reported having been previously bitten by bats (presumably vampires). This indicates nonfatal previous exposure to rabies, including a recent one where the sample had IgM antibodies against rabies lyssavirus ribonucleoprotein, but neither IgG antibodies against this nor neutralising antibodies. As the authors note, these results are difficult to interpret: they could reflect one or more of the effects of multiple bites which individually did not lead to infection; the circulation of a less pathogenic variant in bats in the region; or natural selection of individuals who were resistant to the typical rabies lyssavirus (Gilbert *et al*., 2012).

[56] 0.14-0.66 IU/ml, median 0.18; the generally accepted minimum value for protection in humans is 0.5 IU/ml.

***iii. The Case of Some Spotted Hyenas*** East *et al*. (2001) found evidence that a separate strain of rabies lyssavirus was circulating in spotted hyenas (*C. crocuta*) in the Serengeti National Park in Tanzania, East Africa. In contrast to another population in Ethiopia at least, the strain involved was distinct from that in the park's canids and other carnivorans. About a third of hyenas from three large groups ('clans': Hofer and East, 1995)[57] sampled over a period of 9-13 years were seropositive based on a neutralising test using a WHO rabies standard, with evidence that titres decreased over a period of months. Of these, viral sequences were identified (but no virus isolated) in the saliva of only about a half of individuals, indicating that the others had recovered from prior infection. There was no apparent effect on the long-term survival of animals which had previously tested seropositive. Moreover, although there were no clinical signs of typical rabies infection in any hyenas, brain samples from animals which had died of other causes indicated that about a tenth of these contained evidence for rabies RNA sequences, although again tests with mouse cell-lines proved negative. It was concluded that frequent social interactions – especially involving higher-ranking members – within the large clans, including biting and licking, sustained a non-pathogenic strain of rabies lyssavirus (East *et al*., 2001).

On the other hand, more recent studies have failed to repeat East *et al.*'s findings for *C. crocuta* in the same general area. Thus Lembo *et al.* (2007, 2008; see also Cleaveland *et al*., 2008) found that only site-specific variants of a single strain of the Cosmopolitan clade (Africa 1b) were circulating amongst the diversity of carnivoran species in the Serengeti area (in contrast to the largely species-specific pattern seen in the United States, where there is less species diversity), as a result of dead-end or sputtering spill-overs from dogs, whence the original infection was introduced (Nel and Rupprecht, 2007) and which serve as the maintenance hosts as a result of their rapidly expanding population densities (due to their early reproduction and high birth-rates: Hampson *et al.*, 2009).[58] Of the 41 *C. crocuta* sampled, four were infected with the Africa 1b strain and exhibited typical symptoms of infection; Lembo *et al.* (2007) considered East *et al.*'s (2001) finding of a different strain "difficult to explain", although this

[57] Members of these 'commute' outside their normal home range during the migratory seasons of potential prey in order to forage for food (Hofer and East, 1985).

[58] Rabies was seemingly eliminated as a threat to wildlife on the Serengeti through dog-vaccination programmes between 1954 and 1978 (Cleaveland *et al*., 2008); however the early reproduction and rapid turnover of dog populations means that vaccination drives need to be regularly repeated (Hampson *et al.*, 2009).

might be related to the populations sampled.[59] More recently, a ten-year survey by Prager *et al.* (2012) reached a similar conclusion regarding the role of dogs as a reservoir in northern Kenya. On the other hand, to further confound the picture, a study of otherwise healthy African lions (*Panthera leo*), African wild dogs (*Lycaon pictus*) and *C. crocuta* in two Zambian National Parks found that 40% of lions, but none of the other two species, were positive for rabies virus neutralising antibodies (Berentsen *et al*., 2013).

Other serological studies (Harrison *et al*., 2004) have identified antibodies against canine distemper virus (see also Prager *et al.*, 2012)[60] and a variety of feline viruses in *C. crocuta*. Given their feeding behaviour, which includes scavenging, it might be expected that they would be exposed to infections (or at least subthreshold doses of pathogens) from deceased animals which had escaped being predated upon opportunistically by other species whilst succumbing to rabies or other diseases. Presumably related to this, an analysis of the genes for the major histocompatibility complex[61] in these and striped hyenas found evidence for positive selection of various loci, which was suggested to reflect their feeding on carrion (Califf *et al.*, 2013).

## 6. Conclusions

Stereotypically, rabies and, where studied, other lyssaviruses have the ability to infect terrestrial mammals through a sophisticated mechanism involving taking over the central nervous system and influencing the behavioural output of its present host in order to try to maximise the chances of daughter virions being passed on to new hosts through salivary output downstream of the autonomic nervous system. Thus, for example, a study on raccoons suggested that selection of the bat-derived variant now enzootic in this species may have been related to its pathogenicity (Hamir *et al.*, 1996): animals inoculated with a virus isolated from a conspecific died suddenly or developed more severe 'furious' symptoms than those receiving a Latin American canine isolate, although the latter developed clinical symptoms earlier. This was associated with differences in the evident extent of the central spread of infection when

[59] However one basis might be differences in sampling locations, and the associated influence of population densities for the reservoir of dogs. Thus, in contrast to East *et al.* (see Hofer and East, 1995), Lembo *et al.* sampled spotted hyenas outside the National Park itself, with analyses based on "brain stem samples … collected from suspect rabies cases and carnivore carcasses, whatever the apparent cause of death." Dog densities were highest to the northwest of the Park, in Serengeti district, and lower to the east, in the Ngorongoro district (and presumably lowest in the Park itself), with evidence that the maintenance of rabies depends on host densities being at least 5 dogs/km$^2$ (see also Cleaveland and Dye, 1995), consistent with other studies emphasising densities of dog being the primary factor (Knobel *et al*., 2007).

[60] This virus causes high mortalities in spotted hyena cubs (East *et al*., 2001).

[61] An important initial component of the innate immune system.

animals were sacrificed: whilst those given the canine strain showed evidence for infection of the brainstem, this also included the cerebrum as well as various outputs associated with the autonomic nervous system in those receiving the enzootic strain. It was suggested that the latter strain had evolved to be better able to circumvent its hosts' immune system and thereby establish an ultimately more productive infection (in terms of behavioural as well as virile output), in order to maximise the chances for onward transmission to new host individuals given the pending demise of the existing one. More generally, Hanlon *et al.* (2013; see also *e.g.* Streicker *et al.*, 2010; Mollentze *et al.*, 2014; Marston *et al.*, 2018) have argued that each species-specific strain has evolved to become adapted to their particular host species in terms of being able to establish a productive infection which can be transmitted on to new hosts before the present one succumbs: there is a need to strike a balance between virulence and the potential for onward transmission.

Moreover, different lyssaviruses may use different acceptors in order to gain access to their prefered target cells (Weir *et al.*, 2014). For example, whilst the glycoproteins of the rabies virus and European bat lyssavirus type 2 interact with the low-affinity nerve-growth factor receptor $p75^{NTR}$ (a membrane protein found on many synapses as well as in a variety of other tissues including muscle and salivary glands), this is not the case with those of the Australian bat virus, European bat lyssavirus type 1, Duvenhage virus, Lagos bat virus and Mokola virus (Tuffereau *et al.*, 2001). However, the possible effect of differences in the structure of this neurotrophin receptor in the natural host aside, $p75^{NTR}$ is not essential for successful infection by rabies viruses (Tuffereau *et al.*, 2007). Moreover, the fact that this receptor is not found at the neuromuscular junction suggests that any role it may play in the initial infection of the nervous system is mediated by sensory projections (Lafon, 2005).

Experimental studies involving intramuscular inoculations typically only involve one particular target site for the convenience of the investigators. However, as noted above (Section III.2), both the incidence of natural infections and the speed of development of clinical symptoms would appear to be dependent on the distance of inoculation from the central neuraxis. It is not clear what factors might influence where more distant inocula are more likely to be less effective (Jackson, 2007): one possibility might be related to the relative densities of nicotinic cholinergic receptors in muscle tissues (suggested to be a factor in accounting for species-differences in susceptibility: Baer *et al.*, 1990) as a means for initially boosting the original inoculum.

Studies on naturally-infected dogs indicate that there is the need for these to exceed a certain minimum population density in order to sustain an infection; and that this depends on the behaviour of individual animals (including their human owners in the case of household dogs), both normally and after active infection (Brunker *et al.*, 2012). Whilst a spill-over into red foxes was associated with the appearance of a new strain which initially radiated outwards from its original locus, with die-offs of many in local populations, this was followed after a time-lag by sporadic occurrences in the population in its wake as they recovered in numbers (Wandeler, 2004). The introduction of a typical rabies virus into a fully susceptible new host population would rapidly die out with those it infected where the host population is relatively small and isolated (Bingham, 2005). Presumably the successful establishment of a rabies strain in such a population would require that it be relatively less virulent in the sense that it is less lethal to its new host species, so that a reservoir can be established and maintained: this might be the case for the strain in mongooses on Grenada for example, and also for hyenas in dog-free portions of the Serengeti. Thus the small isolated Afroalpine populations of the Ethiopian wolf (*Canis simensis*) are threatened with extinction[62] partly as a result of the recent spread of the Cosmopolitan strain of the rabies virus (Randall *et al*.. 2004; Nel and Rupprecht, 2007), leading to attempts to vaccinate wild populations as a control measure (Knobel *et al*., 2007).

'Less lethal' might, on the one hand, be a result of (pre-adapted) genetic variability in host susceptibility (Scott and Nel, 2016) and the potential for natural selection for more resistant individuals (and thus either milder or abortive infections). This might be through properties of the innate immune system (Srithayakumar *et al*., 2011, 2014; Kyle *et al*., 2014) or other factors which lead to either a much more prolonged and variable (trans-generational) latent period;[63] or to active infection, but with milder symptoms and subsequent recovery. Where recovery occurs, individuals might transit to become potential carriers if the infection is not completely cleared by the immune system and the virus is able to hide in immune-privileged tissues from where it can re-emerge for potential onward transmission at a later date.

Alternatively, or in addition, 'less lethal' might reflect the particular (sub-) lineage of the virus involved, including its ability (or otherwise) to quell the apoptotic response of the host. Experiments with 'fixed' strains which arise *in vitro* indicate that attenuated versions of the

[62] http://www.iucnredlist.org/details/3748/0: compounding the effects of habitat loss and other more direct human pressures.

[63] Thus, as noted above, infectivityhas been correlated with the relative densities of nicotinic cholinergic receptors in muscle tissue of a particular species (Baer *et al*., 1990), and thus presumably amongst and within individuals depending upon different muscles.

original street virus could potentially evolve and be selected for in such situations; furthermore, the case of the SHBRV suggests that alternative viral strategies have evolved in the wild. This will be considered further in Section V.2.

Although East *et al.*'s study failed to identify actual viruses in the saliva of spotted hyenas, Jackson (2007) considered that this "interesting report really changes our perspective on the ecology of less virulent viral variants and is an exception to the old dogma that rabies virus kills the great majority of exposed individuals. It is likely that in the future we will learn that under some circumstances the situation is similar in other species, including bats." This may include the influence of genetic differences, including for the functioning of the innate immune system (Scott and Nel, 2016). The latter group of mammals is the topic of the next section, where there is also evidence of findings in natural populations reminiscent of the limited studies on mongooses on Grenada and ferret badgers in East Asia.

## IV. Lyssavirus Infections in Bats

As noted above, bats are generally considered to have been the original reservoir, given that molecular techniques have found evidence for a variety of members of the genus *Lyssavirus* in various species of Old World bats. On the other hand, whilst members of the type species *Rabies virus* have generally not been identified in Old World bats, a distinct indigenous American clade has been identified in a diversity of bats in the New World (see Section II).

The following will first consider studies of lyssaviruses circulating in the wild, based on passive and active surveillance techniques; this is followed by a brief review of the results of experimental studies; and then a consideration of how the results of these different approaches have been integrated in an effort to understand the dynamics of these viruses in natural populations.

### 1. Field Evidence for Bat Infections with Rabies and Related Viruses

In the absence of the ideal of isolating the virus itself as evidence for current infections, this may involve the use of antisera to detect the presence of particular viral epitopes (more-or-less specific to particular species of *Lyssavirus*), often in neutralising tests; or, more recently, probing for evidence of particular genomic 'signatures' for one or more of these viruses. Such tests are limited to oropharyngeal swabs and blood samples in cases where bats are released after sampling; otherwise, the brain, salivary glands and perhaps other tissues may also be

sampled. A general problem is that small species will only yield proportionately smaller samples of saliva and blood.

***i. Evidence for 'Clinical' Infections*** The classic approach has been passive surveillance, doing a *post hoc* determination of whether a rabies-related virus may be associated with the deaths of bats in a particular population (*e.g.* Schatz *et al*., 2012). In addition, other indicators might include abnormal behaviours prior to death: as in other mammals, bats terminally infected with rabies may develop a 'paralytic' form of the disease, where they land on any stationary object; or a 'furious' form where they are hypersensitive to environmental stimuli, including approach and handling (Constantine, 1967; Kuzmin and Rupprecht, 2007; Vos *et al*., 2007; Banyard et al., 2011). Thus many cases of human infections in the United States result from attacks by rabid solitary bats, often occuring in response to noise and especially during the day, being swift and unexpected; however the behavioural changes can be difficult to detect clinically, since normal bats are themselves very sensitive to threats (Constantine, 2009). Paralytic infections would seem to be characteristic of gregarious bats in large colonies, whilst the furious form is more associated with solitary species (Kuzmin and Rupprecht, 2007; Constantine 2009; Banyard *et al*., 2011), possibly related to the fact that different clades of virus are enzoonts (Davis *et al*., 2006).

Studies on *Tadarida brasiliensis mexicana* (family Mollosidae),[64] a species which can form huge roosts in caves, indicate that about 10% of sick and dead bats tested positive for rabies, compared with a background infection of 0.1-0.5% in apparently healthy bats (Constantine 2009). On the other hand, about 90% of *T. b. mexicana* found grounded underneath a small bridge roost had detectable rabies virus in their brains (with antigen also being detected in the salivary glands of 69% of these); assays for the presence of neutralising antibodies found these in a proportion of both rabies-positive and -negative bats, and it was concluded that a variety of variants which differed in their pathogenicity were circulating in the population (Davis *et al*., 2012a). Nevertheless, in a review of causes of bat mass-mortalities,[65] O'Shea *et al.* (2016a, b; see also Messenger *et al*., 2003) concluded that there was no definitive evidence for rabies being the underlying cause either of a large die-off of *T. b. mexicana* in Carlsbad Cave in Texas or of mortalities of other colonial bats elsewhere in the United States: if involved, any infection

[64] Two subspecies of the free-tailed bat – *T. b. brasiliensis* and *T. b. mexicana* – have typically been recognised; the latter is found in Mexico and much of the southern and western portions of the United States (Russell *et al.*, 2005; *cf.* http://www.iucnredlist.org/details/full/21314/0).

[65] Defined as "cases in which ≥ 10 dead bats were counted or estimated at a specific location within a maximum timescale of a year, and more typically within a few days or a season".

was likely to be superimposed upon the effects of various environmental stressors (see also Constantine, 2009; Wang *et al.*, 2011). However this lack of evidence for mortalities may reflect sampling problems: Constantine (2009) considered that most rabies cases in bats go unnoticed because they die in sheltered roosts or fall in dense vegetation. Moreover, to save energy when hanging in a roost, the hind feet of bats have a locking mechanism which, in contrast to other mammals but like that of perching birds, keeps their toes flexed around a substrate;[66] thus those which succumb while roosting may be unlikely to drop dead until they have been sufficiently recycled by dermestid beetles and their larvae or other scavengers.

In contrast to the general view that the rabies virus is not found in Old World bats (see Section II.2), Lu *et al.* (2013) found evidence from a survey of sick and dead bats in Guangxi Province in southern China (no information was presented regarding species composition) for isolates related to the standard rabies virus (two of the ten had high homologies with Guangxi street rabies viruses) in 85 of 2,969 bats.[67] However, these data provide no evidence that such infection was the cause of mortality: the fact that only 2.86% were infected suggests that this reflects background rates unrelated to the cause of death.

Regarding other lyssaviruses, the only apparent evidence is for such a die-off in a species of fruit-bat in southern Africa which was associated with infection by Lagos bat lyssavirus (reviewed by O'Shea *et al.*, 2016a).

The results of passive surveillance need to be interpreted with caution, since they depend on a constant level over time. Thus there has been an apparent dramatic increase in reports of rabies-infected bats in the United States; however this reflects increasing public awareness, since the proportion of bats submitted which test positive has remained constant (Constantine 2009).

***ii. Evidence for 'Subclinical' Infections*** This is based on samples of apparently healthy bats from active surveillance of a population. Depending in part on legal restrictions, sampling may be limited to testing blood for (neutralising) antibodies and oropharyngeal swabs for intact virus or target RNA sequences. In cases where the animal is sacrificed, viral tests are also applied for the brain and salivary glands, and also other tissues in some cases. Results may be biased by the size of bats: serum samples are likely to be limited for smaller species.

[66] https://animals.howstuffworks.com/mammals/question668.htm

[67] Inoculation tests in mice were inconclusive, with no evidence for clinical signs of rabies (suggested to be because the short duration of the experiments), although some virus could be detected in response to up to ten blind passages in suckling mice for about a quarter of the extracts tested.

Baer and Smith (1991) have reviewed evidence for the apparent paradox that rates of ongoing infection were lower in gregarious than in solitary species of North American bats. They noted that this could reflect sampling bias as a result of differences in susceptibility to capture but, on the other hand, colonial species had high rates of seroprevalence.

Sampling of apparently healthy bats in North America indicates that the numbers of brain-infected bats showed biennial peaks in migratory species, whereas there was only a single peak in those non-migratory species studied (Kuzmin and Rupprecht, 2007; Constantine 2009). This was superimposed upon evidence for a much higher level of seroprevalence, taken to imply 'failed' infections. Thus, in the case of *T. b. mexicana*, up to 80% may be seropositive, compared with a background infection of 0.1-0.5% in apparently healthy bats. Serological studies on North American bats indicate that transmission rates may often be highest in maternal roosts, suggested to be timed to ensure onward infection of the immunologically-naïve next generation (*e.g.* George *et al*., 2011). Thus Steece and Altenbach (1989) found that, on the one hand, less than 1% of adult females in a maternal roost of *T. b. mexicana* had detectable rabies virus[68] in the brain and only 2% had detectable IgM in their blood compared with 69% for IgG; on the other, 2% of juveniles had detectable virus overall, with a marked peak in August after levels of (presumably maternally-derived) IgG had fallen and associated with the start of a rise in IgM levels; the authors concluded that the juvenile bats thereafter recovered from infection based on the high levels of adult seropositivity.

An extensive survey of seven other species of insectivorous bats – five colonial and two which were solitary and migratory – found varying rates of neutralising seroprevalences in the absence of any detectable virus in oropharyngeal swabs which could not be related to the host species' lifestyles (Bowen *et al*., 2013), despite their viruses belonging to different clades (Davis *et al.*, 2006). It was concluded that exposure to rabies virus is common in diverse bats, with infections leading to an acquired immune response and failing to progress to clinical disease, perhaps as a result of (repeated) exposure to sub-threshold doses.

Two types of European bat lyssavirus have been identified as circulating in western and central areas, with limited overlap in geographic distribution and apparently different reservoir host genera; moreover, there is no evidence that the most abundant bat species in the region (*Pipistrellus pipistrellus*) is a natural host for either virus species (Vos *et al.*, 2007). *Eptesicus*

[68] The enzootic virus is close related to that in *D. rotundus*, somewhat more so than is that of *T. b. brasiliensis* (Kuzmina *et al.*, 2013).

spp. have been identified as the reservoir for the more widely distributed European bat lyssavirus type 1, although the virus has been identified in a variety of other genera of insectivorous bats (reviewed by Banyard *et al*., 2011; McElhinney *et al.*, 2013).[69] Pérez-Jordá *et al*. (1995) showed that there was an annual variation in the presence of antibodies against European bat lyssavirus type 1 in four colonies of *Eptesicus serotinus*[70] in southern Spain, with a post-hibernation spring-time peak of 74% in one of the colonies before rapidly falling back to less than 10% (with only low titres, at most, in tagged individuals). A five-year-long study of 19 maternity colonies of *Ep. isabellinus* in southern Spain (Vázquez-Morón *et al*., 2008) found that the virus was present in 2.8% of 1225 oropharyngeal swabs, with no effect on body condition; one bat remained positive when captured again one week later, whilst another was negative three years later. Antibodies were detected in 9.3% of 549 samples, including in two of 22 bats with RNA-positive swabs. There was little evidence for movements between the 19 colonies and different between-year patterns of circulating virus were seen amongst these, indicating that there were no common underlying epidemiological factors.

A seroprevalence for European bat lyssavirus type 1 has also been found in other Spanish breeding colonies where *Eptesicus* spp. were infrequent (Serra-Cobo *et al*., 2002, 2013; Amengual *et al*., 2007; López-Roig *et al*., 2014); *Mi. schreibersii*, *My. myotis*, *Tadarida teniotis* and *Rhinolophus ferrumequinum* included individuals with a positive antibody response but not any of the other species including *Ep. serotinus* itself. A study of two discrete colonies over 12 years (Amengual *et al*., 2007) found that there was generally a seasonal variation in seroprevalence with a peak in July when maternity colonies typically formed; there was no evidence for gender- or age-related differences. Some individuals were seropositive for more than a year (assuming this was not due to recurrent infections), with 19 bats subsequently becoming seronegative at least one year later. Differences between years in seroprevalence were not associated with evidence for differences in mortality rates. Two of 17 bats found dead were positive for viral nucleoprotein RNA but not for the virus itself. Tests of 426 blood clots for viral nucleoprotein RNA found a prevalence of 4.72%; two of the 20 were also seropositive.

[69] There is evidence that European bat lyssavirus type 1 may also be transmitted to captive collections of African fruit-bats (*Rousettus aegyptiacus*); whilst this was first detected as a result of mortalities, subsequent analyses of the rest indicated that many were seropositive and had presumably survived infection (Ronsholt *et al*., 1998; Wellenberg *et al*., 2002).

[70] This would seem likely to be *Ep. isabellinus* instead: the latter has been distinguished from *Ep. serotinus* based on genomic characteristics (Juste *et al.*, 2013), with a range which encompasses the southern Iberian peninsula and adjoining areas of North Africa (http://www.iucnredlist.org/details/full/85200107/0; *cf.* http://www.iucnredlist.org/details/full/7911/0).

There is also serological evidence for prior infection with another phylogroup I member, Duvenhage virus, in an insectivorous bat (*Nycteris thebaica*)[71] in Swaziland (Markotter *et al.*, 2013).

In phylogroup II, the Lagos bat virus has received most attention. Two species of fruit-bat, *Eidolon helvum* and *Rousettus aegyptiacus*, are likely to be the main reservoir despite the general failure to detect viral RNA in those tissues sampled (see above; Schneeberger and Voigt, 2016),[72] although other species of mainly fruit-bats have also been found to be seropositive (Hayman *et al*., 2008; Dzikwi *et al*., 2010; Kalemba *et al*., 2017). Whilst *Ei. helvum* roosts during the day in trees, the ability of *Ro. aegyptiacus* to echo-locate means that it does so in caves, raising the question of whether and how the virus may be shared between these two species (Kuzmin *et al*., 2008c); one possibility might be that this occurs in so-called 'night roosts' to consume fruit and for pregnant and lactating females to rest (Kunz, 1982).

Most studies have focussed on *Ei. helvum*, a species which is found throughout sub-Saharan Africa and makes migrations – up to more than 2,500 km in about five months (Hayman *et al*., 2012a) – tracking the seasonal abundance of fruit (Richter and Cumming, 2006).[73] Thus there is a continent-wide exchange of genetic information as a result, associated with serological evidence for an exchange of various viruses for this species in Ghana and elsewhere in its range (Hayman *et al*., 2008; Peel *et al*., 2013). In Ghana, Hayman *et al*. (2012b) found a high seroprevalence of antibodies against Lagos bat lyssavirus, more especially in mature bats than in juvenile or sexually immature ones and in females more than males, despite there being no evidence for viral RNA in those tissues sampled.[74] There was no difference in the survival of seropositive and seronegative bats over an 18 month longitudinal study. Similarly, a radio-telemetry study with one female *Ei. helvum* from a roost in Accra which had neutralising antibodies against Lagos bat virus and also (the first report for this species) antibodies against

[71] The species identified as the likely source of a prior human spill-over in Zimbabwe.

[72] Kuzmin *et al*. (2008c) identified the virus in one dead *Ei. helvum* (itself seropositive with virus in the saliva) but not in live samplings of 440 of these and *Ro. aegyptiacus*, where there was a seroprevalence of 40-67% and 29-46% respectively. On the other hand Freuling *et al*., (2015) identified Lagos bat virus lineage A in one of an unstated number of brain samples of *Ei. helvum* from Ghana without evident signs of disease; virus neutralization tests using the isolate with sera from the same group of bats found a seroprevalence of 74% for 567 animals, in a greater proportion of adults than in juveniles.

[73] http://www.discoverwildlife.com/animals/fruit-bats-africas-greatest-mammal-migration

[74] Wright *et al*. (2010) found that, of the 56% of *Ei. helvum* which had virus-neutralising activity against Lagos bat virus in Ghana, a large proportion also had such activity for Mokola virus whilst an additional smaller number were also seropositive for West Caucasian bat virus instead; however they considered that this was most likely due to crossreactivity.

*Zaire ebolavirus* found that the bat, having left the area in the meantime during the wet season, returned and survived for at least 13 months before apparently leaving again at the start of the next wet season (Hayman *et al*., 2010). Hayman *et al*. (2012b) concluded that viral infection may occur seasonally and/or elsewhere, perhaps related to reproduction and migration to unknown maternity roosts; and that viral infection may persist in another, untested (possibly immune-privileged) tissue. A subsequent study by Suu-Ire *et al*. (2017) found that there was a continued high seroprevalence for neutralising antibodies, although they found no difference between juveniles and adults,[75] or between wet and dry season samples. Other such studies affirm that infection is not generally associated with mortality, including evidence for sustainable viral circulation in an isolated island population (Peel *et al*., 2013).

With regard to the Shimoni bat virus in Kenya, a relatively high rate of seroprevalence was found in the insectivorous hipposiderid *H. commersoni* (from which the virus had previously been isolated: Kuzmin *et al*., 2010), as well as in a higher proportion of two of the three species of fruit-bat tested, *Ro. aegyptiacus* (which roosted in the same caves) and *Ei. helvum* (Kuzmin *et al*., 2011). In contrast, only a small proportion of *H. commersoni* were seropositive for Lagos bat virus, compared with almost half of each of the two species of fruit-bat; interpretation of the results is complicated by some degree of cross-neutralisation of sera between the two viruses, reflecting their relatively close relationship. There is also serological evidence that Shimoni virus is one of the variety of lyssaviruses circulating amongst bats in Nigeria (Kia *et al*., 2014).

Similarly, there is serological evidence for West Caucasian bat virus or a related phylogroup III species in *Miniopterus* spp. in East Africa (Kuzmin et al., 2008d).

Although no lyssaviruses have been isolated from Southeast Asian bats or those in southern China,[76] with only potential partial sequences in a study of the latter (Wu *et al*., 2015a, b), there is serological evidence of prior exposure to lyssaviruses in fruit-bats from Cambodia (Reynes *et al.*, 2004) and these and other bats elsewhere in the region (reviewed by Banyard *et al*., 2014a; A. D. Munro, in preparation). Whilst two humans in northeast China died from a rabies-type disease which apparently spilled over from bats, this is likely to have been from the Irkut bat lyssavirus, another phylogroup I species isolated from '*Murina leucogaster*' there and in eastern Russia (Wang *et al.*, 2014).

[75] Note that there is a typographical mistake in their Table 2.
[76] With one apparent exception of the rabies virus, noted above: Lu *et al.* (2013).

Where tested, virus has been detected in tissues other than the brain and salivary glands. Constantine (1967: cited by Kuzmin and Rupprecht, 2007) isolated rabies virus from various tissue samples from 130 naturally-infected *T. b. mexicana* collected from Texan caves: whilst all of those from the brain had detectable virus, positive results were also obtained for salivary glands (79%), lungs (30%) and kidneys (12%); other tissues were tested for a subset of 50 of these, where virus was detected in brown fat in two whereas liver, spleen, pectoral muscle, intestines and faecal pellets were all negative. Other studies include one of naturally-infected vampire bats in northeastern Brazil, where viral RNA was found in lung tissues, as well as the uteri and placentae of some pregnant females (Carneiro *et al*., 2010). More recently, a sample of 26 non-hematophagous bats (half of them *Artibeus lituratus*, a frugivore; the rest were various species of insectivorous bat) submitted for testing in São Paulo state found that, apart from all brain and salivary gland samples being positive with PCR analyses for a vampire bat-related rabies virus, all other tissues tested were also positive in some individuals, with some evidence for differences between *A. lituratus* and the others (Allendorf *et al*., 2012). Thus a high proportion of positive results (> 60%) was obtained for samples of the tongue and the lungs; for the stomach and, in *A. lituratus*, the intestines (*cf.* 40% in the faeces of these and the other bats); and for the bladder and, in *A. lituratus*, the kidney (*cf.* 40% for other bats).

Regarding other lyssaviruses, there is growing evidence that the tongue may be a major target in naturally-infected bats based on PCR analyses, as found by Allendorf *et al*. (2012). Amongst other phylogroup I viruses, there is evidence for European bat lyssavirus type 1 in various species (Serra-Cobo *et al.*, 2002); and for type 2 in naturally-infected *My. daubentonii* (Johnson *et al*., 2006a). Similarly, in group II, high concentrations of Lagos bat virus were detected in the tongue of a rabid *Ei. helvum*, as well as in oral and nasal swabs (Kuzmin *et al*., 2008). In anticipation of the following section, experimental infection of *Ep. serotinus* with European bat lyssavirus type 1a resulted in evidence for the infection of the taste buds and associated epithelial cells as an alternative route for viral shedding after intracranial injection in a bat which died of infection (Freuling *et al*., 2009); it is intriguing to speculate that this might also be an alternative means for infection in the first place and, if so, could even serve as a positive feedback loop to boost infections when viral excretion by the salivary glands is low.

Concerning the urinary system in naturally-infected bats, there is evidence amongst other phylogroup I viruses for European bat lyssavirus type 2 in the bladder of *My. daubentonii* (Johnson *et al*., 2006a). For Lagos bat virus (group II), antigen was restricted to the innervation of the bladder of an *Ei. helvum*, considered to exclude the urine as a route for viral excretion

(Kuzmin *et al*., 2008). In the case of group III viruses, there was evidence for Bokeloh bat lyssavirus in the kidney and bladder of *My. nattereri*, although only as trace amounts of viral RNA (Picard-Meyer *et al*., 2013).

Regarding the lungs, there is evidence amongst other phylogroup I members for European bat lyssavirus type 1 in these and the upper respiratory tract in various species as a result of natural infection (Serra-Cobo *et al.*, 2002). In the case of group III viruses, there was trace evidence for Bokeloh bat lyssavirus in *My. nattereri* (Picard-Meyer *et al*., 2013).

**2. Experimental Studies with Rabies and Related Virus**es **in Bats**

Caveats considered above regarding *in vivo* studies on terrestrial mammals (see Section III.4) apply equally to bats; there would appear to be no *in vitro* studies. Thus differences in dosage of viruses, together with their origin and prior passage history in tissue culture, without any standardisation, confound making any but the broadest generalisations; as does the history of the bats being tested and whether they had previously been exposed to virus in the wild (Banyard *et al*., 2011). Also, as noted above, there is the problem of interpreting serological studies in the absence of any rigorous standardisation of assay protocols to make it possible to compare results from different laboratories (Baker *et al.*, 2014; see also *e.g.* Freuling *et al*., 2009, 2015).

Moreover, there is the need for caution in interpretation, since the bats may be stressed due to the unnatural holding and associated husbandry conditions (Constantine, 1967, 2009; Freuling *et al*., 2009), although the latter authors suggested that the regular provision of food under captive conditions would alleviate stress for laboratory-adapted animals. Based on laboratory studies with plasma glucocorticosteroids as the proxy for assessing stress, circulating basal hormone levels were unusually high in established captive colonies of two species of megachiropteran (Old World) fruit-bats tested as compared with three species of neotropical microchiropterans, comprising captive colonies of two species of frugivore and one species of insectivore sampled in the wild (Widmaier *et al.*, 1994); levels were further elevated by handling stress, together with that of adrenocorticotropin (ACTH: a major regulatory control of their secretion, with pre-stress levels which were typical for other mammals) in the fruit-bat *Pt. hypomelanus*. In the absence of information regarding the feeding of captive colonies, it is not clear whether elevated levels of corticosteroids are the result of a generalised stress response or perhaps problems with the availability of amino acids and the need for catabolic

recycling.[77] In turn, activation of the pituitary-adrenocortical axis might be expected to impair immune function, unless compensatory countermeasures are taken.

Experimental studies on the effects in Brazilian vampire bats of a rabies virus, isolated from a naturally-infected conspecific and injected intramuscularly, indicated that there was evidence for a dose-dependent effect on bat mortality over a tenfold stepwise range from $10^2$-$10^5$ $MICLD_{50}$ (mouse intracerebral lethal dose for 50% mortality), with the lowest being ineffective and the highest leading to six of ten recipients succumbing; survival times for those which died were negatively correlated with dose (Almeida *et al*, 2005). Rabies virus was detected in all of the ten bats which died (eight as a result of the paralytic form, with none showing the furious one),[78] as well as in the salivary glands of 40-60% of them, the latter depending on the virus test-procedure used. All apart from one of the survivors on the lowest dose showed virus-neutralising activity on day 30 – by which time all of the highest dose mortalities (6/10), as well as half of each of those resulting from the two intermediate doses (1/10), had occurred – which decreased thereafter with evidence for a variability in antibody response not related to the original dosage.

Another study, on vampires from Mexico (Aguilar-Setien *et al*., 2005), reported that 11 of 14 bats receiving a higher dose ($10^6$ $MICLD_{50}$) of another vampire bat variant succumbed within 30 days of inoculation without any evidence for virus in their saliva; on the other hand, the other three remained healthy for at least two years thereafter, with virus being transiently isolated from the saliva early on – once in two at six days after injection and at 21 days in the third.[79] The authors suggested that, in the lack of any knowledge about the history of the recipients, the survivors might have benefited from an anamnesistic response to a previous infection.

Vampire bat variants have also been used to determine their infectiveness in other bats, as presumed by some hypotheses regarding the origins of indigenous American strains. Thus, for

[77] Studies on vampire bats caught in the wild indicate relatively high 'baseline' levels of glucorticoids (the duration of time in the net before sampling is a confounding factor) and a very high responsiveness of these to ACTH compared with a frugivorous species of phyllostomid bat and other mammals, which was suggested to be diet-related as a result of the need for *D. rotundus* to mobilise reserves at times when foraging proves suboptimal (Lewanzik *et al.*, 2012); this is over and above evidence for genomic and microbiomic evidence for positive selection for the ability to handle the problems associated with their nutrient-poor, potentially pathogen-rich diet in the first place (Zepeda-Mendoza *et al.*, 2018).

[78] Symptoms were only manifest after the affected individual was isolated.

[79] Saliva samples of all living bats were taken daily for the first 30 days, then weekly for two months, and finally one and two years later.

example, the virus when tested in *Artibeus intermedius* (a phyllostomid which is frugivorous) was found to lead to the death of only one of three bats inoculated with an intermediate dose intramuscularly, with no evidence for behavioural or neurological changes; surprisingly, none of the three receiving the same dose intracranially succumbed (Obregón-Morales *et al*., 2017). Surviving bats which received intramuscular or subcutaneous injections showed a transient seroconversion.

Interpretation of the results of experiments with North American bats would also seem to be complicated: that there is not a simple all-or-nothing response has also been reported from studies on North American bats with regard to sero-conversion and/or the development of manifest disease. In part, this may reflect differences in the mode of administration: intracerebral injections of lyssavirus are more commonly associated with pathologic effects, compared to other more natural forms of administration to the periphery (reviewed by McColl *et al.*, 2000; Banyard *et al.*, 2011, 2014a).

Thus, for example, an early study on *T. b. mexicana* (Baer and Bales, 1967) found that the effects of exposure to virus from a rabid conspecific varied with the mode of administration. Compared with intracerebral injection, intramuscular, subcutaneous and intranasal routes were less effective in terms of resulting mortalities, with the anomalous result of one bat receiving the lowest of the four doses by the last procedure; moreover it was the only other bat to manifest the 'furious' behaviours seen in those which succumbed after intracerebral injections. With regard to mortalities, there was evidence for a spread of when these occurred, being up to six months (when one survivor was found to have antigen in its brain, as well as in those other organs tested without any evidence for neutralising antibodies: it is not clear how bat #17 had been treated). There was evidence for salivary virus-excretion in all bats which died, as well as one of the bats which survived after receiving the lower intramuscular dose other tissues which included evident rabies virus were the brown fat, kidneys, and lungs. Neutralising antibodies were detected in six of the 52 which survived, including two which received intracranial injections.

In a study on the little brown bat (*My. lucifugus*), Davis *et al.* (2013a) reported that the primary inoculation of an enzootic strain, one (*Efv2*) from *Ep. fuscus* or SHBRV into the deltoid muscle led to a more rapid onset of clinical disease than was the case for overlying subcutaneous injections for those bats which developed clinical disease, although the two receiving injections by the latter route which developed clinical symptoms were the only ones to intermittently shed virus in their saliva for up to 18 days previously.

In the case of the big brown bat (*Ep. fuscus*), tests on a captive colony of 38 bats with 20 receiving an intramuscular injection of a viral isolate from a conspecific and comprising 11 groups with 1-3 uninfected and infected members (Jackson *et al*., 2008) found that the mean incubation period was 24 days (range 13-52 days, N = 16), with the three which succumbed first failing to seroconvert. Salivary excretion of virus was detected in only two individuals, and there was no evidence for onward transmission to the controls. The four survivors had detectable antibody titres which peaked within the first two months, thereafter declining to below threshold by the end of the 140-day experiment.

Another experiment with this species compared the effects after intramuscular injection of two variants – *Efv1* and *Efv2* – isolated from rabid conspecifics (Davis *et al*., 2013b). Mortalities associated with paralytic-like behaviour were only seen with the second viral variant, with six of the 11 either succumbing or being put down; interestingly,[80] however, two of the six receiving the higher dose recovered from their rabies-like clinical symptoms as a result of supportive care (hand-feeding, *etc.*) and moreover these were two of the four which tested positive for virus in their oral swabs in the meantime. Like most of those receiving *Efv1*, *Efv2* induced a neutralising antibody response all recipient bats apart from one of those which died; based on monthly samples, antibody titres often showed an initial increase before thereafter generally declining. Another group of bats, comprising five which had been seropositive for neutralising antibodies at the time of capture six months prior to the start of the experiment, survived inoculation with the higher dose of *Efv2*; whilst they had become seronegative by the start of the experiment, three of the five showed a marked increase in titres one month later (Davis *et al*., 2013b).

Other studies on *Ep. fuscus* indicated that there was a relationship between the effects of initial exposure to an enzootic variant of the rabies virus and whether there was a pathological response after intramuscular injection (Turmelle *et al*., 2010a): mortalities were where there was a reduced immune response (as assessed by the development of neutralising antibodies) and were independent of the dosage administered. Thereafter, when surviving bats were given a second injection 175 days later with the highest dose, there were comparable levels of mortality; a further challenge of the remaining survivors with the highest dose another 130 days subsequently was associated with reduced mortalities. Monitoring of titres of neutralising antibodies indicated that these were elevated transiently after inoculation; thus it was proposed

[80] The authors caution not to read too much into this!

that susceptibility may be reduced by repeated infections as a result of anamnestic immunological responses (Turmelle *et al*., 2010a). The transient nature of the experimentally-induced immune response contrasts with evidence that naturally-infected *Ep. fuscus* may maintain prolonged elevations of neutralising antibodies (Shankar *et al.*, 2004). To further confuse things, there may be differences between species in the nature of experimentally-induced acquired immune response, as suggested by a study on *T. b. mexicana* (Turmelle *et al*., 2010b) where a commercial monovalent inactivated rabies vaccine induced the production of neutralising antibodies which remained in a proportion of bats one year later.[81]

In an experiment comparing the effects of intramuscular injection with either of two variants of the fixed CVS-24 strain in the frugivorous *Artibeus jamaicensis*, it was found that one of these led to pronounced neurologic symptoms (*cf.* paralytic rabies) which were associated with a much more widespread infection of the brain (with no evidence for apoptosis) as compared with the other where clinical effects were not apparent (Reid and Jackson, 2001); unfortunately there is no mention of salivary levels, presumably due to the experiment being designed .to track the short-term time course of the respective variants on the progress of brain infection.

As more realistic than intranasal administration, Davis *et al.* (2007) tested the effects of exposure to aerosols with isolates of viral strains from *Ep. fuscus* (*Efv1*), *T. b. mexicana* or *L. noctivagans* (SHBRV) on *T. b. mexicana* and *Ep. fuscus* (four of each species for each strain). All bats developed a neutralising immune response after a single 80-minute exposure. Whereas four of nine mice exposed to similar aerosol regimes at the same time succumbed (see above), there were no mortalities over the subsequent six months; however an intramuscular challenge with *Efv2* from *Ep. fuscus* at this time led to half of each species developing paralytic rabies. The authors speculated that, although the bats had been quarantined for 6-8 months prior to the experiment, they may have been previously exposed and thus sensitised to rabies viruses (see also Davis *et al.*, 2013c).

Experimental studies with the enzootic Australian bat lyssavirus or a variant of the rabies virus from *Ep. fuscus* in the fruit-bat *Pteropus poliocephalus* after intramuscular injections found comparable effects. A proportion of bats died after either virus; whilst the latter was detected in the brain, none was found in the saliva, possibly because the affected bats were sacrificed soon after the appearance of clinical symptoms. There was variability in the extent of development of an immune response, with sero-conversion only seen in a proportion of those

[81] The presence of an adjuvant may have led to a more pronounced immune response, however.

which did not manifest clinical symptoms by the time the experiment was terminated three months later (McColl *et al*., 2002).

Banyard *et al*. (2011) have summarised experimental studies on Eurasian lyssaviruses. In the case of European bat lyssavirus type 1a, subcutaneous injections were associated with higher mortalities than by way of the intramuscular route in *Ep. serotinus*, with one bat shedding virus in its saliva (Freuling *et al*., 2009); somewhat similar results were found for European bat lyssavirus type 2 in *My. daubentonii* (Johnson *et al*., 2008). Immunohistochemical studies of an *Ep. serotinus* which died after intracranial inoculation of European bat lyssavirus type 1a identified viral antigen not only in various areas of the brain but also throughout the spinal cord including in dorsal and ventral horn neurones; as well as in autonomic ganglia and the innervation of the tongue and salivary glands, but not in the secretory or ductal epithelial cells of the latter (Freuling *et al*., 2009). Intriguingly, as noted in the foregoing section, the profuse infection of the taste buds together with the associated epithelium of the tongue suggests not only a possible route for viral shedding but also for initial infection and/or as a positive feedback route.

Testing the European bat lyssavirus type 1 in the North American *Ep. fuscus* led to mortalities after intramuscular injections, with latency being shorter for the higher dose; no mortalities were seen after intradermal administration (*via* scratches on the leading edges of the wings: associated with problems of ensuring a standardised dosage) or after intranasal or intra-oral inoculation (Franka *et al*., 2008). Behavioural changes preceding death included evidence for furious rabies in some cases. There was a transient seroconversion after the intramuscular and intradermal protocols, with a more rapid decline for the latter, but not with intranasal or intra-oral administration; seroconversion was also seen in bats which subsequently died. Virus was occasionally detected in the saliva of bats which subsequently died, as well as the salivary glands, the oropharyngeal area, the lungs, brown fat, pectoral muscle, kidney and bladder of some *post mortem*; on the other hand, it was also detected in the saliva of one bat 28 days after receiving a high dose intramuscularly which survived for the remaining 63 days but did not seroconvert (Franka *et al*., 2008).

Three other members of phylogroup I, the Aravan, Khujand and Irkut viruses from eastern Eurasia, have been tested in *Ep. fuscus* from North America (Hughes *et al.*, 2006). Mortalities were moderate, with virus not spreading to co-habiting uninjected bats, despite sporadic isolation of virus from the salivary glands and oral swabs within five days of detectable clinical disease. Apart from the brain, Irkut virus RNA was also detected in the salivary glands, brown

fat, lungs, bladder and, to a lesser extent the kidneys; on the other hand, that for the Aravan and Khujand viruses was mainly found only in the brain.

In the case of phylogroup III, Kuzmin *et al.* (2008e [abstract]; see also Banyard *et al*., 2011) reported that experimental infection of 21 *Ep. fuscus* with West Caucasian bat virus led to the deaths of three of 15 animals inoculated intramuscularly between 10 and 18 days later; the remainder survived until the experiment was terminated after six months (with four having sustained seroconversion), as did all six where the virus was administered orally. Apart from the fact that the subjects were North American bats, interpretation of these results is further confounded by the fact that some had been used in previous studies of the Irkut virus and still had neutralising antibodies against the latter 12 months later, at the end of the subsequent experiment.

### 3. The Dynamics of Lyssavirus Transmission in Bats

Various authors have noted that, where information is available, the circulation pattern of lyssaviruses in bats would appear to be different from those described in most carnivoran studies (*e.g.* Kuzmin and Rupprecht, 2007). Moreover, there is evidence for differences between colonial and solitary North American species with regards to the pathogenicity of enzootic strains: gregarious species appear to be less susceptible to infection than solitary ones and thereafter manifest mainly paralytic symptoms, in contrast to the latters' furious presentation (Kuzmin and Rupprecht, 2007; Constantine 2009; Banyard *et al*., 2011).

As briefly reviewed in Section IV.1, several studies on wild colonies of North American and European bats have linked peaks in transmission to the seasonal establishment of maternity roosts and the influx of immunologically-naïve young, once their maternally-derived passive immunity has worn off (*e.g.* Steece and Altenbach, 1989). Constantine (1986) failed to find evidence for foetal infections in 15 dams of *T. b. mexicana* or smaller numbers of four others submitted for inspection, although subsequent studies based on the establishment of a foetal cell-line from the first of these species suggest that infection may occur *in utero* but be below the threshold for detection in the assays being used at the time (Steece and Calisher, 1989; the rabies status of the mothers was not tested, although they were "clinically normal").

***i. Background Modelling Studies*** Theoretical analyses by Dimitrov *et al.* (2007, 2008; Dimitrov and Hallam, 2009) were based on the overall results of surveys of *T. b. mexicana* in North America, a migratory bat which summers in often vast colonies in the southwestern United States, where there is a marked disparity between viral prevalence (1-4%) and a much

higher neutralising seroprevalence (up to 65% or more) (see also Section IV.1.ii). Dimitrov *et al*. (2007) proposed an individual-based immune response model where bats infected with a dose below a particular survival threshold value were able to develop an acquired immune response which eliminated most of the virus; thus they survived infection, in contrast to those receiving supra-threshold inocula. According to their model, the waning of antibody levels in the survivors thereafter led to the recrudescence of the virus and thus of circulating antibody titres in an oscillatory fashion which could potential allow intermittent onward transmission of infection, provided that residual virus remained after the bat's original infection.

Subsequently, Dimitrov *et al.* (2007, 2008; Dimitrov and Hallam, 2009) developed a new class of 'adaptive epizootic models' to analyse and thereafter make predictions, based on the evident variation in intra-host viral dynamics within bat populations as a result of between-individual diversity. On the one hand, this may reflect differences between individuals in the inoculum dose(s) received and the consequent infectivity of these; in part, this will depend on differences in the adaptive (acquired) immune responses of individual bats, given that large inocula can pre-empt the development of the latter.[82] Their model also included an 'impulsive' birth mechanism[83] whereby the seasonal production of young led to the emergence of a naïve population. Subsequent models incorporated not only different ecotypes[84] (referring to individuals originating from different geographical areas, and thus different experiences of disease) but also the impact of diversity, leading to the identification of various 'immuno-types', based on differing levels of immunocompetence.

However, as Dimitrov *et al*. (2007; see also Rupprecht *et al*., 2011) note, a major (potential) issue is that there is no evidence for bats being able to act as infectious carriers of rabies virus in this way, although it may apply for other diseases where there is evidence for this. This will be considered further in Section V.

***ii. Vampire Bats*** The impact of rabies spill-overs from *D. rotundus* on the cattle industry in Argentina stimulated an early interest in this field. This provided evidence for seasonal outbreaks of rabies related to roosting and migratory behaviour associated with reproductive

[82] The authors excluded initial susceptibility and the associated role of the innate immune system (reflecting the evolutionary history of individual bat's ancestral lineages and the consequences of past positive selection processes) due to a paucity of information regarding the latter.

[83] *i.e.* one where there is a periodic driver for the resurgence of infection, once any maternally-derived immunity has worn off.

[84] An ecotype comprises individuals which have identical characteristics relative to a particular environment; immune-types are one subcategory, based on the categorisation of overall differences in immune responses.

cyclicity and the resultant peaks in numbers of immunologically naïve young bats associated with seasonal rainfall (Lord, 1980, 1992; see also review by Blackwood *et al*., 2013a). Superimposed on this was the longer-term spread of zoonotic outbreaks along the course of rivers in South America, with evidence that rabies lyssavirus may be a 'migratory epizootic'.[85] Thus there has been evidence for the virus spreading southwards as a wave in northern Argentina, to infect immunologically naïve colonies at a rate of about 40 km/year; about 24% of bats were virus-positive on the crest of the wave, with associated spillings-over into cattle (Lord, 1980). The spread was mainly along rivers (see also Kobayashi *et al.*, 2008) or *via* roosts at waterholes. About the same percentage of bats were seropositive in its wake, with varying percentages (including some high ones) of virus-positive bats also being found at various times thereafter; presumably the latter serve as the origin of another wave about four or more years later, when enough immunologically-naïve new bats have entered the population. As a result, extermination of bats in area 30 by 50 km and sealing off their previous water-hole roosts interrupted the advance of a wave of rabies in cattle which had been advancing for the previous 14 years, although it did proceed onwards in the area to the west (Fornes *et al.*, 1974). Lord (1980) refers to similar migratory waves of rabies elsewhere in South America, including an epidemic on the island of Trinidad between 1923 and 1937 which originated in large towns and then spread southwards to progressively smaller ones; evidence implicated bats for its passage, with no evidence for introduced Indian mongooses being involved (Mungrue and Mahabir, 2011; Seetahal *et al*., 2017).

Later studies in the same area of Argentina (reviewed by Streicker *et al*., 2012c) indicated that such a migratory pattern had been replaced during the period 1984-1993 by the virus circulating locally. Thus Torres *et al.* (2014) reported that, rather than periodic eruptions, there was the continuous threat of bovine rabies during the period 1991-2009, as a result of local enzootic cycles with variable persistence. Similarly, a long-term study of the same species of bat in Peru (where rabies is an emerging infectious disease) found no evidence for such a wave-like spread based on serological studies (Streicker *et al*., 2012c; Blackwood *et al*., 2013a, b; Condori-Condori *et al.*, 2013); instead, there was a sustained circulation within each of the four regions sampled over a period exceeding three years, although it varied over time. Seroprevalence was highest in juvenile and sub-adult bats with no decline during the first year of life and was unlikely to be as a result of maternally-derived antibodies; it was suggested that infection

[85] Reminiscent of the 'wave hypothesis' as one possible explanation for the spread of *Zaire ebolavirus* as seen in African great apes (Walsh *et al*., 2005; Biek *et al*., 2006; see also Quammen, 2014).

contributed to the 50% mortality rate during this time (Streicker *et al*., 2012). Presumably there was an initial migratory component leading to the recent invasion of the disease across the Andes from the Amazon basin as a result of movements of males between roosts, females being more philopatric and thus constituting genetically-isolated populations (see also Huguin *et al*., 2017); modelling analyses projected that the virus would reach the Peruvian coast by 2020 (Streicker *et al*., 2016). Streicker *et al*. (2012c) concluded that there was no evidence for population density thresholds for viral invasion and extinction, with exposure independent of bat colony size: they suggested that frequency-dependent transmission reflected the fact that bats are limited in the number of neighbours that they can bite (or groom: Lord, 1980), which would seem to assume that each bat occupies the same resting site over time in a roost (which might be reasonable in large colonies, given the evidence for reciprocal altruism: Wilkinson *et al.*, 2016).[86]

Using simulations based on the findings of Streicker *et al*. (2012c) and the assumption that transmission was frequency- rather than density-dependent, Blackwood *et al*. (2013a, b; see also Johnson *et al.*, 2014) concluded that viral persistence in vampire bats was dependent on two factors: a high frequency of nonlethal immunising infections (estimated to represent about 90% of all infections), together with the movement of infectious bats between roosts as a local metapopulation. The simplest susceptible-exposed-infected-recovered model (I) was found to best fit the field observations, resting on the underlying assumption that there was a spatial asynchrony in infectivity between roosts in a region: exposed bats either develop a non-immunising[87] infection (subsequently leading to onward transmission of the virus) which is always lethal or enter a non-infectious state with temporary immunity (Blackwood *et al*., 2013a). One of the three alternatives (model IV), which presumed life-long immunity to abortive infections, was also dependent on immigration but fitted the observational data only poorly. The remaining two models, which postulated either recovery from a transient infectious state (model II) or immune-boosting as a result of increasing force of infection and the resultant delayed return to a non-immune state (model III), were found to be possible, albeit less robust alternatives. This model contrasts with that proposed to underlie the presumed mechanism

[86] This reflects the fact that, apart from water, ingested blood is only high in protein, with the need to convert the latter for short-term energy production rather than use food-derived carbohydrate reserves for a longer-term insurance (Naish, 2007a), which presumably acts as a constraint to render these bats relatively sedentary.

[87] This assumes a situation similar to dogs and humans, for example; however various studies in bats suggest that an acquired immune response may develop in at least some species (including vampires) prior to the development of classical clinical symptoms (see Section IV.2).

underlying the epizootic wave in that the latter was (assumed to be?) driven by mortalities during its passage.

***iii. Big Brown Bats*** The growing appreciation that spill-overs of particular strains of rabies virus from North American bats pose a potential risk to humans has also led to many experimental studies, as briefly reviewed in Section II.2. The big brown bat (*Ep. fuscus*) is not a major source of human spill-overs (Messenger *et al.*, 2003) but provides a useful model for the characterisation of the infection dynamics within a closed population of bats, although the situation is complicated by the presence of at least two variants – *Efv1* and *Efv2* – with only the latter leading to clinical effects and mortalities after intramuscular injection (Davis *et al*., 2013b). The data from one extensive five-year study on a non-migratory population of *Ep. fuscus* in Colorado were used by George *et al*. (2011) to compile a model to investigate the possible means by which this virus may circulate and be maintained. It was assumed that infection was lethal to the bats; thus those with rabies neutralising antibodies were deemed to have been exposed to an abortive infection, rather than being recovered from a (subclinical) infection. They proposed that hibernation and facultative torpor at other times, by reducing not only a bat's immune activity but also viral replication rates, allowed the average incubation period of an infection to be prolonged so that the virus is more likely to be sustainable in the population through transmission to the young in maternity colonies the following year. Runs of the model where there was no hibernation period (such as would be the case for tropical bats) consistently led to high mortalities, especially amongst juveniles, and population crashes. Their models also indicated the importance of viral incubation period for sustainable infectivity: too short a time meant that there was likely to be epizootic fade-out prior to hibernation due to reduced chances of transmission after bats have dispersed.

Based on serological data from a number of urban maternity roosts of *Ep. fuscus* in Colorado over a period of up to five years, O'Shea *et al.* (2014a) found that a greater proportion of adult females (up to almost half in some roosts) had neutralising antibodies than did males, which they related to the former tending to roost more closely together. Seroprevalence also increased with age, being lowest in juveniles, apart from those which presumably had passive immunity from their mothers, and one-year-old males. Survivorship of marked individuals was not affected by whether they were seropositive or not, with some later becoming seronegative: infections were presumed to be abortive, given that no rabies virus was detected in swabs or post mortem samples of seropositive bats.

Logistic regression analysis failed to find any relationship with female reproductive condition, the number of fresh puncture holes in the wing that might represent recent bites from other bats, days elapsed since prior sampling or the mean minimum and maximum ambient temperatures for the five preceding days (O'Shea *et al.*, 2014a). The transition of adult females from seropositive to seronegative was best explained by a model based on the year of sampling, being more likely in 2004 and 2005 as compared with 2003; whereas the opposite transition was reflected by an evident progressive intra-seasonal spread of (abortive) infections within the individual roosts as a result of (density-dependent) contagion. Moreover there was an interaction between the year of sampling and the within-season day of sampling which influenced the likelihood of an adult female being seropositive at first capture, associated with a consistent pattern across adults in all five of the roosts sampled over the period 2001-2005, with the frequency of seroprevalence being lowest in a drought year with high local insecticide use (2004) and highest in a more 'normal' year (2003). O'Shea *et al.* (2014a) proposed that that the differences between 2003 and 2004, in particular, might be explained by levels of environmental stress, such that increases in the latter may lead to the depression of antibody production (in terms of amplitude and/or duration), presumably in the face of a constant potential infection pressure. Whilst they cite Constantine's (1967, 2009) previous ideas as being consistent with this hypothesis, it is important to note an important difference: the latter author was speculating about the occurrence of active rather than abortive infections, with stress leading to infection-mediated mortalities during migrations of the likes of *T. b. mexicana*, presumably as an impairment of overall immune function.

These authors went on to compare their field observations with the extensive experimental findings from their and others' laboratories on the same species (briefly reviewed above in Section IV.2). They noted the consistent evidence that seropositivity may wane over time, although a residual anamnestic response, seen in laboratory populations, may serve to protect against exposure to future infections (and thus complicate the interpretation of experimental studies with wild-caught bats of unknown exposure-history: Davis *et al.*, 2013c). They further suggested that 'stress' from decreased food availability due to changes in potential prey populations may attenuate the trajectory of immune responses to viral exposure, at least in part through the pituitary-mediated production of glucocorticosteroids.

***iv. Other Lyssaviruses*** As considered above (Section IV.2), infections with European bat lyssavirus type 1 have been found to have a seasonal component based on studies in Spain (where bats do not hibernate), primarily of mixed maternity roosts of what is considered to be

the main reservoir host (*Eptesicus* spp.) as well as other species. Amengual *et al*. (2007) considered that infection was in the process of becoming established in one of two colonies they studied, associated with evident oscillations in the low levels of seroprevalence and positive blood-clots between successive years, but that these had stabilised at relatively high levels in the other; based on data for the latter, they were able to estimate the host population fraction that was susceptible at equilibrium and thence derived a value for the basic reproductive number (their '$R_0$') of 1.706.[88]

Based on these and follow-up studies, Serra-Cobo *et al*. (2013) found that overall seroprevalences varied between 3 and 37% amongst 68% of 25 sampled roosts,[89] with neutralising antibodies in 13 of the 20 species from the four different families sampled, wherein seroprevalence varied between 11.1 and 40.2%.[90] Of 45 dead bats, 12 were positive for viral RNA, in six out of seven species; whilst 2% of blood-clots were positive from six out of the seven species where such samples were analysed. They used the seroprevalence data for the period April to October (when bats were more active and infection rates were higher) and five potential explanatory variables (taxon, month, sex, colony size and species richness) to determine possible ecological drivers for infection using a generalised linear-mixed model. The four variables other than sex explained more than 90% of the variability. Based on this analysis, July was the peak month, when maternity colonies were mostly present; vespertilionids were most likely, compared with members of the families Miniopteridae and, especially, Molossidae and Rhinolophidae, perhaps reflecting differences in susceptibility to initial infection and/or the nature of the subsequent immunological response in relation to phylogenetic distance. Also, seroprevalence was higher in larger colonies, perhaps related to host density and contact rates; moreover the impact of colony size was increased if it included at least three different species, suggesting that density-dependent increases may involve other dimensions (including the import of virus by migratory bat species, for example).

[88] The average number of other bats a productively-infected bat would be expected to infect in a *wholly susceptible* (*i.e.* immunologically naïve) population (Viana *et al.*, 2014). For comparison with rabies virus in dogs, Hampson *et al.* (2009) found an $R_0$ of ≈ 1.2 from a study in areas adjoining the Serengeti National Park in Tanzania and values of less than 2.0 elsewhere in its range, using different models. However, it should be noted that this parameter depends on the assumption that all members of a particular population have not been previously exposed to the contagion in question – something questionable in the case of the Balearic bats, at least – and that the susceptible population is homogeneous, rather than being a metapopulation comprising different species with (presumably) different pre-adaptations of their innate immune systems.

[89] In northeastern Spain and the Balearic Islands.

[90] The latter figure for 270 *My. myotis*; *cf.* 16.4% for 19 *Ep. serotinus*.

A subsequent analysis of these serological data over the period 1995-2011for three maternity roosts on the Balearic islands with mixed bat populations[91] found high levels of seroprevalence for European bat Lyssavirus type 1 (Pons-Salort *et al*., 2014); the fact that the same viral sequences were identified on both islands indicated that there was an exchange between the three metapopulations surveyed, without any input from *Ep. serotinus* (generally considered to be a normal reservoir), and no evidence for contact with the Spanish peninsula. The model assumed that infection was seasonal and frequency-dependent, leading to transient rather than life-long immunity without any increase in mortality. As with the original sampling data, this suggested that, rather than persisting in the long-term in each of the metapopulations, there were fade-outs at various times followed by viral re-introduction in the absence of *Eptisecus* spp.; *Mi. schreibersii* emerged as being the likely local reservoir host, despite *My. myotis* being twice as abundant.

## 4. Conclusions

Field evidence indicates that only a small proportion of a bat population is likely to be actively infected with a lyssavirus at any one time, based mainly on the analysis of brain and salivary samples; whereas there is evidence of often widespread past infections (abortive or otherwise) based on seroprevalence studies. Experimental studies using other than the intracranial route for administration give an inconsistent picture with regard to survivorship and (transient) seroconversion (reviewed by McColl *et al*., 2000; Turmelle *et al*., 2010a); it has been proposed that the high seroprevalence rates against lyssaviruses in certain wild populations of bats suggest that either many bats have recovered from a prior clinical infection or they have been exposed (possibly repeatedly) to subclinical infections which were abortive due to a pre-emptive immune response and thus presumably failed to spread to infect the nervous system (reviewed by Banyard *et al*., 2011, 2014a; Barrett, 2011; Kuzmin *et al*., 2011). Thus the riddle appears to distil down to, as Banyard *et al*. (2011) put it, "Bat lyssavirus serology: Infection or exposure?" Furthermore, exposure of whatever sort may be even higher: Banyard *et al*. (2011) have argued that the use of neutralisation tests to identify whether bats had been exposed might be too rigorous, especially given the findings of Turmelle *et al*. (2010a) regarding transient albeit anamnestic responses; they suggested that the use of more general tests (*e.g*. ELISA-based) for rabies-related antibodies be also used to screen potential experimental subjects (see also Moore and Hanlon, 2010; Moore *et al.*, 2017).

[91] *Mi. schreibersii*, *My. capaccinii*, *My. myotis* and *R. ferrumequinum.*

Studies on the development of rabies infections in bats are necessarily long-term, largely resting on the presumption that the sole mechanism for establishment and onward transmission is the same as has been documented for 'classic' rabies infections in 'typical' terrestrial mammals. However, the potentially 'premature' termination of many experimental infections may mean that the normal progression towards the full-blown manifestation of such clinical symptoms may be pre-empted (Banyard *et al.*, 2011), especially if infective cycles have apparently evolved to coincide with an annual pattern of host reproductive activity (George *et al.*, 2011). As will be considered further in Section V.3, a variable, often prolonged latency is but one dimension of the potential hypothetical range of actions of this and other lyssaviruses in bats.

Vos *et al.* (2007) have noted that, in contrast to the indigenous American clade of the rabies virus and its promiscuous spread amongst microchiropteran bats across the continent (with spill-overs into terrestrial carnivorans), the two types of European bat lyssavirus are much more species-specific with regard to their natural hosts: most notable is that there has been no evidence for natural infections by either of one of the most abundant bat species in the region (*Pipistrellus pipistrellus*), despite extensive surveys. They postulated that, by adapting to one particular set of reservoir species through reducing their virulence and pathogenicity (and thus the impact their infections on the host population), they reduced their capacity to infect other, more distantly related bats (see also Section II.3).

The epizootic migratory pattern for the initial establishment of rabies infections as it expanded its geographical range in vampire bats is reminiscent of that seen for the westward spread of a variant of the Cosmopolitan strain in twentieth century foxes in Europe, where the lull in its wake was attributed to high host mortalities; there were oscillations in the disease's prevalence over subsequent years (Wandeler, 2004). Such a cyclicity has also been reported after sustained spill-overs of rabies into various North American mesocarnivorans (other canids, skunks and raccoons) leading to damped oscillations of epidemics alternating with inter-epidemics (reflecting those in local population densities); ultimately this leads to a low background of sporadic disease outbreaks (Childs and Real, 2007), and thus the likelihood of the infection dying out.

George *et al.* (2011) developed a model for *Ep. fuscus*, a non-migratory North American bat with a marked seasonal cyclicity, which was based on the assumption that infection with the enzootic rabies strain was terminal; the latency between initial infection and the development of clinical disease was sufficiently long in order to match the annual cycle of host reproduction

and the appearance of the next cohort of naïve young as potential replacement hosts. This assumption is compatible with studies on rabies in canids and certain other terrestrial carnivorans; however, superimposed on this is the need for outbreaks to be tied to an externally-driven annual breeding pattern. Thus modelling suggested that the fact that this species undergoes hibernation, with a slowing down of metabolism which acts as a brake on viral replication, might help to ensure such an infective cyclicity: this thus represents a special case, not applicable to bats resident at lower latitudes or those which migrate there to over-winter.

Studies on other bats have also proposed that the production of a new cohort of bats is related to the sustenance of lyssavirus infections. However they do not presume that infections are necessarily terminal, but instead that at least a proportion of those bats infected recover, based on the results of various experiments involving extra-cranial inoculation. This has formed the basis for models of the dynamics of rabies in vampire bats and those of European bat lyssavirus type 1 in Spanish populations of insectivorous bats. Thus, for example, instead of the damped oscillations seen in certain terrestrial carnivorans (Childs and Real, 2007), Amengual *et al*. (2007) considered that the opposite occurred in their study of two Spanish roosts: that there was a trend towards rising to a plateau over the long-term. Such sustained circulation within a population clearly requires 'carriers', whether as a result of wide variation in the latency of initial infection (following the standard model for terrestrial mammals: see Section III.2) or due to virus remaining resident thereafter in the brain and/or other immune-privileged sites. Contrariwise, it is not clear why the other roost was apparently in the initial stages of establishing a new enzootic infection: one possibility (Bingham, 2005) is that the virus had previously been maintained in this roost but had died out as a result of the build-up of herd-immunity, with viral persistence in the regional metapopulation as a whole leading to re-introduction through movements of infected individuals between roosts.

In considering why a strain of rabies virus might become established in particular terrestrial carnivores, Wandeler (2004) noted that not only population densities (such that the host carrying capacity exceeds a critical threshold: Childs and Real, 2007) and characteristics regarding innate sensitivity and the potential to generate a sufficiently productive salivary output were likely to be of importance; in addition, general characteristics of the host species are also likely to be an additional determinant, given the assumption that an infection is invariably fatal. Thus, like dogs, hosts of derived strains not only have a wide distribution with relatively high population densities (helping to establish a spill-over in the first place) but also are opportunistic, with relatively short life-spans and high reproductive rates as a consequence:

thus local populations can rapidly rebound after a serious infection. This may pattern of damped oscillations may be modulated by individual differences in the properties of the innate immune system and thus resistance to infection, based on short-term evidence on evolutionary time-scales (Srithayakumar *et al*., 2011, 2014; Kyle *et al*., 2014; Scott and Nel, 2016).

However, as pointed out by Wandeler (2004), this contrasts with the life-history characteristics of bats: they are what has traditionally been termed *K*-selected (Crichton and Krutzsch, 2000; Begon *et al.*, 2006). Thus their volant life-style limits the number of offspring that a female can carry at one time (both during pregnancy and during the subsequent period up to weaning) and, presumably as a result, they have anomalously long life-spans for their body-weights, associated with biochemical mechanisms to limit the effects of free-oxygen radicals generated during the extreme exertion associated with flight (Zhang *et al.*, 2012). Thus Messenger *et al*. (2003) and others have noted that the *K*-selected reproductive strategy (late maturity and low fecundity) of bats implies the rapid weeding out of viruses which have severe effects on the individual; and/or that bats may rely on alternative, possibly novel defense strategies to rapidly contain infections received from other members of a roost or colony (see also *e.g.* Kuzmin *et al*., 2011; Olival *et al*., 2012, 2015).

This sets the scene for the following section.

## V. Discussion

The nature of infection with *Rabies virus* would seem to pose puzzling aspects. Whilst the virus has dramatic and drastic effects in its main terrestrial reservoir host (dogs) as well as when these pass it on to certain other earthbound animals (including humans), there is evidence that various strains may exist in bats and at least some non-volant mammals (including dogs) which are less pathogenic. This poses the riddle of how such seemingly less virulent infections might be maintained in their host populations where there are no profound terminal changes in virion production (based on salivary samples) with associated changes in host behaviour in order to facilitate the likelihood of onward transmission. On the other hand, there is the broader enigma of how lyssaviruses might have originated in the first place, as presumed spill-overs from arthropods. The subsequent realisation that the cross-over from arthropods may have been first to bats would seem to have done little to clarify things, arguably because it is generally assumed that rabies and related viruses have the same profile of actions in these as in other mammals.

Viral infections may be either acute, where they are short-lived due to the host either dying as a result or the infection itself dying out due to the actions of the host's immune system after a

transient transmissive phase; or they may be chronic, whereby the virus can persist in the host in a dynamic and metastable equilibrium as a result of measures taken by the virus to avoid sterilising immunity and its being purged by the erstwhile host as a result, whilst also minimising its destructive effects on infected cells (Virgin *et al.*, 2009), with various alternative strategies being used by particular viruses (Nathanson and Gonzalez-Scarano, 2016). Classic rabies infections[92] exemplify a third strategy, whereby an initial 'smouldering' infection (Nathanson and Gonzalez-Scarano, 2016) leads to a delayed acute transmissive phase after a variable latent period.

In being neuro-invasive, classic rabies infections – and thus lyssavirus infections in general, it would seem to be generally assumed – bear various similarities to those of α-herpesviruses in humans. The following will consider the latter as one possible model of a 'primitive' strategy for the initial productive infection of the nervous system, such as might have led to the progressive evolution of dumb and paralytic rabies in a 'typical' terrestrial host mammal. Thereafter, in order to address the question of how lyssaviruses as a genus may have first evolved in bats, various seemingly relevant aspects of the latters' biology will be briefly reviewed, mainly with regard to how this may play a role in the transmission of viruses from one host to another, with particular regard to these rhabdoviruses. Finally, one possible model for the evolution of lyssaviruses, culminating in that of the rabies virus, will be suggested in the light of these considerations.

### 1. A Comparison of the Strategies of the Rabies Virus and Selected α-Herpesviruses

Most studies have been on the *Rabies virus* in relation to the expression of the classical aetiologies of furious and paralytic rabies, leading to the conclusion that this virus is extraordinarily well adapted to infection of the mammalian nervous system (Lafon, 2016). As such, studies in terrestrial mammals have started to unravel the sophisticated means by which it has the capacity to propagate within its existing host and maximise its potential to spread to other hosts thereafter. Thus, as reviewed in Section III, after peripheral inoculation and possibly initial amplification in muscle, it migrates centripetally to enter and thereafter spread within the central nervous system, to subsequently extend its reach centrifugally to infect at least some non-neural tissues, most notably the salivary glands as the source of output of virions for potential onward transmission. The latter process is facilitated by the virus also acting on the

[92] *i.e.* those manifest as either furious or paralytic cases.

brain itself to induce changes in behavioural output, especially in the case of furious rabies (Marston *et al.*, 2018).

Such an all-encompassing action involving the brain is in marked contrast to what is known of other viruses which infect the nervous system. One prototypical example is the pattern of infection seen with human α-herpesviruses, represented by human herpes simplex viruses 1 and 2 together with herpes virus 3 (varicella-zoster virus) (reviewed by *e.g.* Eshleman *et al.*, 2011; Schmidt-Chanasit and Sauerbrei, 2011; Bloom and Dhummakupt, 2016; Nathanson and Gonzalez-Scarano, 2016; Zerboni and Arvin, 2016). These double-stranded DNA viruses are characterised by having two infective phases where contagion can be passed on to others: an initial one shortly after the individual is first infected, before the virus takes up residence as a latent infection in the nuclei of cells in privileged tissues to evade the host's developing immune response; and a potential later one after the infection has been reactivated in the latter tissues. During the initial acute infective phase, the virus can spread into new hosts through the lytic infection of epithelial cells and the release of virions in mucous secretions (including in aerosols) as well as with the subsequent development of rashes in the case of herpes virus 3.[93] It also spreads within the existing host by invading sensory (including olfactory) and autonomic nerve terminals in the infected dermatomes, migrating retrogradely to establish a latent infection in their centrally-located neurone somata; thereafter, there may be restricted local onward transmission to other cells therein, although this is generally very limited (reviewed by Mori *et al.*, 2005; Eshleman *et al.*, 2011; Ugolini, 2011;[94] Koyuncu *et al.*, 2013; Nathanson and Gonzalez-Scarano, 2016). At some indeterminate later date, the host's depressed immune function (including as a result of ageing) and/or various stressors may lead to reactivation of the virus which then spreads back down to the self-same nerve terminals to re-infect the tissues they innervate and cause a productive lytic infection of the latter; in rare cases, a reactivation encephalitis may develop where the virus spreads elsewhere in the central nervous system. Thus the overall strategy is to, in the short-term, spread infection within an immunologically-naïve cohort and thereafter become dormant in them, through refugia in privileged tissues;

[93] In contrast to the local effects of simplex viruses, after initially infecting mucosal epithelia, herpes 3 becomes established in dendritic cells of the peripheral innate immune system which migrate to the lymph nodes, to then pass virus on to lymphocytes, some of which migrate to the skin and thereby infect dermal cells in various areas of the body as well as becoming latent in the associated sensory cells after retrograde transport. The infection of immune cells is presumably mediated by the formation of immune complexes of circulating virions with antibodies which are subsequently sequestered by these cells to release the active virus particles (Nathanson and Gonzalez-Scarano, 2016).

[94] Based on studies in mice with strains of human herpes simplex viruses.

thereafter, if signals from the current host suggest a potential threat to its viability (and thus that of the virions themselves), the virus reactivates and initiates a new infective phase in order to spread, ideally within the next generation of immunologically-naïve recipients. In this way, these viruses can maintain sustainable infections over the long-term in small, relatively closed populations, circumventing the problem of acquired immunity: thus they are considered ancestral human diseases, dating back to the common ancestors of the prosimian lineage or before, and thereafter co-evolving and thus diverging from those shared with other members of the anthropoid lineage (Wertheim *et al*., 2014).

Such co-evolution and the associated species-specificity contrasts with the promiscuous nature of the rabies virus and its ability to infect members of virtually all groups of eutherian mammals to a greater or lesser extent (Hanlon *et al.*, 2013), albeit with evidence for subsequent minor selection of particular biotypes in those species where it is able to establish a chain of transmission (see Section II.2). Apart from this, a comparison of the 'herpes strategy' with that of classic rabies indicates two major fundamental differences. First, if there is any initial amplification of the rabies virus, it is confined to the host itself, in the inoculated muscle of terrestrial mammals at least; in contrast, the initial infection with α-herpesviruses can also be amplified by spreading to other new hosts.

The other basic difference relates to the nature of the latent period and the neural circuits involved. On the one hand, α-herpesviruses typically infect only those neurones innervating the originally infected area; thereafter, reactivation is a simple 'reflex' response to particular signals from the host which might portend the latter's imminent demise (*e.g.* as a result of physiological or psychological stressors): it is essentially a passive process, in the sense that it is host-driven and involves a closed group of neurones, with the original retrograde flow of virions being reversed within the infected neurones to an anterograde one (indeed, 'reflux' is an equally apt descriptor). In contrast, classic rabies infection of a terrestrial mammal is active, in the sense that the virus takes over the host in order to try to gain access to new hosts (most especially in 'furious' rabies), albeit at the expense of the life of its current one. Thus the rabies virus has evolved the means to not only establish a foothold in the immunologically-privileged (Wekerle, 2006; Kaplan and Niederkorn, 2007) central nervous system but also to then spread between neurones therein and ascend through retrograde (dendro-axonal) transmission up *via* higher-order networks to subvert circuits in the brain itself, together with spreading to output targets, all towards the virus' own ends. In order to do so, the rabies virus has had to acquire the means not only to enter neurones and subsequent downstream targets (*viz*. the salivary gland

acini, at least) through the appropriate acceptor molecules; but also other strategies to evade detection by those innate immune mechanisms functioning in neurones (Lafon, 2008, 2011; Johnson *et al.*, 2010; Scott and Nel, 2016; Zhang *et al*., 2016) in order to replicate for onward transmission across synapses. Furthermore, if detected by the innate immune system of neurones, the virus has acquired the means to short-circuit the resulting neuro-inflammatory response through mechanisms to induce apoptosis in any T cells which move in to try to destroy the infected cells (Lafon, 2008, 2011; Scott and Nel, 2016; Zhang *et al*., 2016).

It is obvious that such an overarching strategy, where the rabies virus essentially commandeers the host's brain towards its own ends, is highly unlikely to have spontaneously evolved *de novo*: there must have been intermediate stages in its progress from an ancestral condition, presumably in arthropods, to the present-day highly derived strategy. Furthermore, the promiscuous nature of the rabies virus' potential infectivity means that evolution has somehow acted to favour mechanisms which are common to all eutherians, down to the details at the molecular level for a variety of tissues, presumably favoured by using host acceptor molecules (such as N-CAM) which have been highly conserved during evolution. If this extends to include the phenotypes of similar molecules in other animal groups (such as arthropods), this will enhance the chances of successful spill-overs. In part, such evident flexibility has been suggested to be mediated by 'quasi-quasispecies' properties that the rabies virus manifests *in vitro* (Morimoto *et al*., 1998; see Section III.3.ii).

Given that lyssaviruses as a genus are generally thought to have originated in bats, the following section will consider various aspects of the biology of the latter which may be relevant to trying to get to grips with the problem of how this group of rhabdovirids originated in the first place and thereafter became established as enzootic in mammals.

**2. Aspects of Bat Biology Pertinent to Their Potential Role as Disease Reservoirs**

As reviewed in more detail elsewhere (*e.g.* Munro, 2017 and in preparation), there is evidence that bats may have been the original hosts for many different groups of viruses, with spill-overs leading to the establishment of derived lineages in other mammalian groups.

Bats (order Chiroptera) have an ancient evolutionary history (Bininda-Emonds *et al.*, 2007; Zhang *et al.*, 2012). Today, they form most of the 'night-shift' amongst flying vertebrates, taking over from the mainly diurnal birds. They sleep during the day, often through hanging

upside down by their hind-legs either in solitary locations[95] or in often large roosts (Kunz, 1982); the latter will favour sharing of contagions on a local basis. At night, they disperse to forage, potentially sharing such diseases more widely, especially if individuals move between roosts; such pathogen-sharing may be even more widespread in those species which undergo seasonal migrations in order to track changes in food availability (Rupprecht *et al*., 2011).

One suborder (the Megachiroptera; fruit-bats)[96] is restricted to lower latitudes in the Old World; as their common name suggests, they feed on fruit, albeit with evidence that they may supplement their protein-intake through also eating arthropods (Courts, 1998).[97] Most species rely mainly on vision for navigation, and thus roost on trees or other structures in the open; where studied, *Rousettus* spp. have evolved a means of echo-location which allows them to roost in caves instead. The other, much larger suborder, the Microchiroptera, use a different means for echo-location; whilst the majority are insectivorous, others have evolved a diversity of foraging strategies, including haematophagy in the vampire bats.

As noted in Section II.2, strains of *Rabies virus* were likely to have been circulating in bats and also the Arctic fox and other mesocarnivores in the Americas before the arrival of European colonialists. One possibility is that, like the Cosmopolitan strain, the indigenous American strain was also introduced by dogs accompanying humans during the original colonisation of the New World. According to this scenario, spill-overs would include to vampire bats or their haematophagous ancestors, which then served as the vehicle to initiate the spread of infection to other bats as well as terrestrial mammals. Phylogenomic analyses aside, this presumes that rabies virus circulated in the blood of the imported prehistoric dogs. Whilst it has been concluded that rabies virus does not circulate in the blood of naturally infected humans (Hemachudha *et al*., 2002) or dogs (Sitprija *et al*., 2003), parabiotic and other experimental studies on terrestrial mammals have yielded confusing results in this regard (reviewed by Lodmell *et al.*, 2006), perhaps partly related to whether 'street' or 'fixed' strains were used. Some of these studies suggest that viraemia may be predisposed in animals with depressed

[95] Note that these come together seasonally in mating and maternity roosts and, in some cases, to hibernate; in some vespertilionids, at least, maternity roosts (crèches) and hibernating roosts may be in different locales, with males establishing lek-like territories around the latter (*e.g.* Naish, 2011b).

[96] For simplicity, the traditional subordinal classification scheme has been used here, although it is generally considered to be out-dated.

[97] Assuming that they have not evolved to have reduced dietary protein requirements (Mqokeli and Downs, 2014); at least some species of neotropical frugivorous bats may also be dependent on insects to supplement protein intake and/or to tide over seasonal changes in the availability of their normal food source (Herrera *et al.*, 2002).

immune function; however the presence of neutralising antibodies need not preclude detection of the virus (or at least its RNA).

On the other hand, Serra-Cobo *et al*. (2013) identified *European bat lyssavirus type 1* RNA in blood clots from samples of various bat species in roosts in northern Spain;[98] this suggests that, in principle, other lyssaviruses including the rabies virus might also circulate in the blood. Apart from the potential to spread passively through wounds as a result of fighting, with the ingestion of infected blood, this suggests the potential for spread *via* haematophages, whether through arthropods as vectors where viraemia is high enough or by way of vampire bats.[99] Assuming for the moment that the virus can be transmitted amongst mammals *via* the ingestion of blood, the fact that vampire bats may share blood-meals back at the roost with those which were not successful in their night's foraging (Wilkinson *et al.*, 2016)[100] means that such reciprocal-altruistic behaviour most likely shares any pathogens as a downside, and thus would reinforce the spill-over of the rabies virus and other pathogens. That rabies can cause infection through ingestion is indicated by the fact that skunks and red foxes were found to be relatively susceptible to being fed rabies-infected mice, whereas ferrets and cats were resistant (Bell and Moore, 1971; Ramsden and Johnson, 1975), leading to the development of techniques for the oral vaccination of dogs and other carnivorans (Knobel *et al.*, 2013). It is not clear whether infection may be through local damage in the mouth area or can be effected further down the digestive tract (Jackson, 2007): whilst chicken heads are one potential bait using attenuated strains, minced meat is another (WHO, 2007), implying any wounds must have been pre-existing for these modified live vaccines at least.

However if it is assumed (but see Davis *et al.*, 2006) that the rabies virus lineage originated in bats, as is presumed to be the case for other lyssaviruses, a more parsimonious scenario is that there has been a single set of bat-to-terrestrial mammal set of spill-overs without any subsequent spill-backs. This would also be more consistent with phylogenomic analyses, which indicate that the indigenous American clade[101] shared a common ancestor with a lineage which eventually gave rise to all the other clades of *Rabies virus* (Troupin *et al.*, 2016). Rupprecht *et*

[98] It was not stated whether the bats involved were also seropositive.

[99] Given their need for large blood-meals, it would seem unlikely that vampire bats actively predate upon other bats.

[100] Thus these are the only truly social bats, so far as is known; other colonial species are only gregarious.

[101] With members of group IV, found in vampire bats and the migratory mollosid *T. brasiliensis*, basal to the three groups found in other New World bats (see Section II.2.ii). The Molossidae likely immigrated from Africa with the splitting of Gondwanaland; the origins of the endemic family Phyllostomidae (including vampire bats) are less clear (Arita *et al.*, 2014).

*al.* (2017) and Marston *et al.* (2018) favour the vehicle being a vespertilionid, implying this was from Eurasia into the future North American landmass (see Section II.3). Thereafter, assuming no role for arthropod vectors, the virus must have spread from the original vespertilionid host to other bats of the same and various other families, including to the ancestors of present-day vampire bats. The most likely source of such trans-specific spread would seem to be through through the 'squabbles' characteristic of bat roosts, mixed-species or otherwise; this is explored further in Section V.3.

In the New World, spill-overs of the indigenous American strain have also occurred from bats into terrestrial mammals. In the case of those from vampires, this is presumably through the classic saliva-mediated route. In their feeding on terrestrial mammals, these bats target exposed areas with a rich blood-supply[102] and make superficial bites (5 mm round *x* 5 mm deep: Fenton, 1992). This contrasts with the generally deeper infective bites of *e.g.* dogs, which determines their efficacy (see Section III.1). This suggests that, unless strains of the vampire virus resemble SHBRV (see Section III.3.ii) in their capacity to infect superficial cells, many opportunities for transmission are likely to be abortive if they cannot bypass the immune system: this might explain evidence for seroprevalence in indigenous people (see Section III.5.ii).[103]

However spill-overs of the indigenous American strain into terrestrial mammals have been found outside the range of vampire bats, implicating other species of bat; also, such spill-overs of other species of lyssavirus have also been recorded in the Old World, where there are no haematophagous bats. How these occurred will be considered further below, in reviewing other relevant aspects of the biology of bats.

Amongst mammals, wings are peculiar to bats; they constitute about 80% of the surface area of an African fruit-bat, *Epomophorus wahlbergi* (Makanya and Mortola, 2007); when roosting, they are wrapped around the 'front' of the bat, and thus still present a relatively large surface to the outside (Figures 1 and 2). They thus represent a considerable target for bites during squabbles in the roost, whether driven by a rabies-like infection or for other reasons; moreover, the fur typically covering the most of the rest of the body apart from the face and adjoining regions of the head, together with the small teeth of most bats (*e.g.* Feder *et al.*, 1997), likely restricts the ability to inflict deep wounds elsewhere.

[102] Thus they have sensors for infra-red radiation on their snouts, and then shave the overlying hair in order to target well-vascularised areas before directing superficial bites and lapping up the blood oozing as a result of their saliva containing an anticoagulant (Naish, 2007a).

[103] Contrariwise, this might be counterbalanced if there is the potential for a SHBRV-like capacity for blood-mediated infection.

***Figure 1*** Cave-roosting lesser horseshoe bats (*Rhinolophus hipposideros*);[104] the two bats on the right have covered their eyes to sleep (some species which roost in exposed locations have evolved transparent windows in their wings so that they can monitor for predators without having to move their wings when they wake up).

The main wing membrane can be divided into 3 subsections (Figure 2): the protopatagium extends from the shoulder to the thumb along the leading edge of the wing; the plagiopatagium extends laterally from the torso and hind leg to the fifth phalange; whilst the dactylopatagium (or chiropatagium) occupies the distal portions of the wing between the second and fifth phalanges. In addition, there may be an interfemoral membrane (the uropatagium), which extends between the hind limb and the tail in many bats.[105] Overall, the membrane comprises a thin bilayered epidermis separated by a core of dermal connective tissue with a hypodermis containing a vascular capillary network which has been proposed to be important in gaseous exchange (Makanya and Mortola, 2007), as well as for thermoregulation (Lyman, 1982).[106] Skeletal and intrinsic (intra-membranous) striated muscles, with their associated cranial and cervical innervation for the control of flight and manoeuvrability (Swartz *et al.*, 2012;

[104] Photo by Jessicajil (https://www.flickr.com/photos/69439747@N00/3206961624), downloaded from Google Commons.

[105] The uropatagium is not present in all bats, and is reduced in free-tail bats (*e.g. Tadarida* spp.).

[106] Both as a blanket and as a radiator, as a result of vasodilation (they may also lick saliva on themselves as an additional aid to cooling), over and above the implications of the wings' increased surface area for heat- and evaporative water-loss *vs.* respiratory exchange (and thus the potential for stress-related effects in suboptimal environments, whether on a daily basis or in the longer term). Such conflicting consequences may reinforce the need to enter into torpor during roosting in many bat species, with implications not only for the capacity for their immune responsiveness but also for the potential infectivity of thermo-sensitive pathogens.

Hedenström and Johansson, 2015; Swartz and Konow, 2015), are restricted to the protopatagium and plagiopatagium (Crowley and Hall, 1994; Cheney *et al.*, 2014; Cheney, 2015), as well as the uropatagium (Vaughan, 1970a; Norberg, 1972; Kobayashi, 2017). Apart from this motor innervation and that of the vascular system, there is also innervation of the sensory Merkel cells associated with many of the sparsely-distributed hair follicles, together with other more extensively-distributed somatosensory elements related to the detection of touch and air-flow, including on the dactylopatagium (Sterbing-d'Angelo *et al.*, 2011; Marshall *et al.*, 2015; Swartz and Konow, 2015).

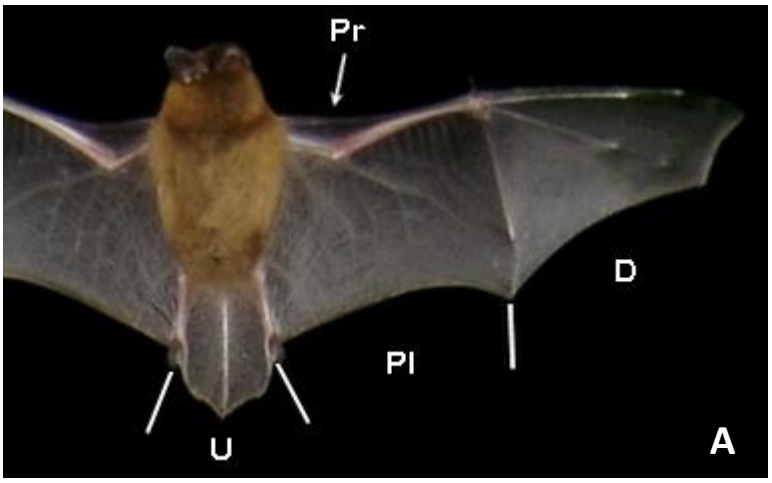
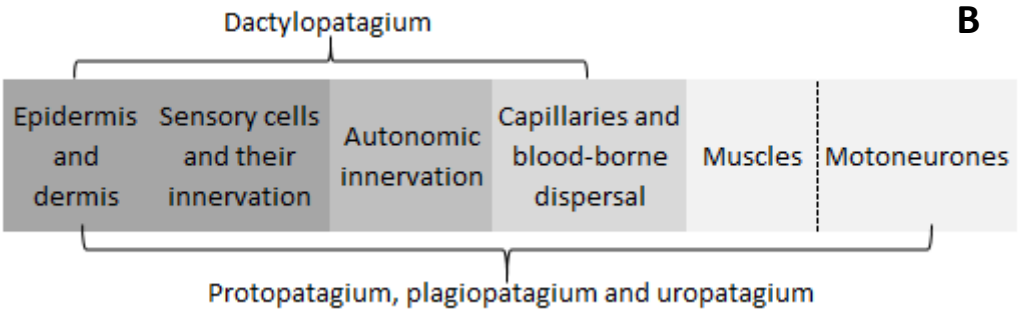



***Figure 2*** *A*: The expanded wings of *P. pipistrellus*.[107] **D**, dactylopatagium; **Pl**, plagiopatagium; **P**r, protopatagium; and **U**, uropatagium; structures visible in the Pl are mainly bundles of elastin fibres. *B*: Distribution of the potential targets for lyssavirus or other infections in different areas of the bat wing.




Differences in vascularisation may mean that the innate immune system is less readily activated in the dactylopatagium compared to the uropatagium, which has been suggested to explain the more rapid wound-healing in the latter as a result of its richer blood-supply and a more effective initial inflammatory response (Faure *et al.*, 2009; see also Weaver *et al.* [2009] for the

[107] Adapted from a photo by Barracuda1983 (https://commons.wikimedia.org/wiki/File:Pipistrellus_flight2.jpg).

plagiopatagium): presumably the thin nature of most of the wing membrane means that direct gaseous exchange with the atmosphere reduces the need for a capillary supply in those areas lacking muscles, although this is at the expense of the other roles mediated by the circulatory system.[108]

Figure 2B summarises the distribution of different tissues in the typical bat wing, and thus the various opportunities for viruses to infect not only intrinsic cell-types in the wing itself but also components of its innervation, as well as entering the blood-stream. In the case of the wing muscles, their nicotininic receptors provide the opportunity for an initial 'booster' to the original inoculation; speculatively (see Section III.2), lower receptor densities may mean that there is a prolonged latency before prodromal and thence clinical symptoms become manifest, assuming a classical pattern of rabies infection.

Assuming that oral secretions are the main medium for viral transmission, other possibilities present themselves, again not restricted to vampire bats. One alternative dimension is suggested by the fact that, as noted above, many species of bat use intense high-frequency sounds for echo-location in order to navigate within their roosts and when foraging outside, as well as to convey information about the sex and other individual characteristics of the emitter (*e.g.* Voigt-Heucke *et al.*, 2010; Jones and Siemers, 2011; Knörnschild *et al.*, 2012). In addition, they also use lower frequency sounds ('social calls') in order to communicate between mother and offspring and during agonistic and other encounters (Fenton, 2003; Pfalzer and Kusch, 2003; Dechmann and Safi, 2005; Luo *et al.*, 2017). Whilst there is evidence that lyssaviruses are detectable in the lungs of at least some bats (see Section IV.2), it is not known whether this is due to infection of the autonomic innervation or is more extensive.[109] However it is conceivable that vocalising could lead to the production of infective aerosols, from saliva at least. If so, inhalation of air-borne viruses would have the potential for relatively direct access to the brain *via* the olfactory system (Winkler, 1968; Constantine *et al.*, 1972; Winkler *et al.*, 1972; Johnson *et al.*, 2006b; Davis *et al.*, 2007), although the results of *in vivo* studies are ambiguous about the potential importance of this route for rabies and other lyssaviruses (see Section IV.2). Nonetheless, reminiscent of a major route for the spread of human α-herpesviruses elsewhere

[108] Apart from the needs of the muscles in the uropatagium as an aid to manoeuvrability, the richer vascularisation of this portion of the wing presumably reflects its being thicker as an adaptation for capturing and propelling prey towards the mouth in insectivorous bats (Swartz *et al.*, 1996; Bullen and McKenzie, 2001; Hedenström and Johansson, 2015).

[109] It would seem unlikely that rabies-related or other viruses induce cold-like symptoms in bats, since developing even minor sniffles would impair the ability to sonicate and thus to navigate in order to find the food essential for short-term survival in many species.

(reviewed by *e.g.* Mori *et al.*, 2005; Eshleman *et al.*, 2011), there is circumstantial evidence that rabies may be transmitted between bats (*T. b. mexicana*) through such aerosols in large cave colonies, where there is a dense congregation in an enclosed space; moreover, foxes, coyotes and hamsters kept in close-meshed cages underneath went on to develop furious rabies after a variable incubation period. In addition, there is evidence for aerosol transmission from laboratory-based studies (reviewed by Constantine, 2009; Banyard *et al.*, 2011), so that it has also been suggested that this route may also account for spill-overs from bats to humans (Conomy *et al.*, 1977; Afshar, 1979); however it is generally considered that so-called 'cryptic' cases[110] are more likely to be through unnoticed superficial bites, although how these lead to productive infection is not known (Gibbons, 2002; Messenger *et al*., 2003; Kuzmin and Rupprecht, 2007; Warrell, 2009). Thus, regardless of the possible mode of transmission, the Advisory Committee on Immunization Practices in the United States has recommended that post-exposure prophylaxis should be given in cases of direct contact with a potentially rabid bat when a bite or scratch cannot be ruled out (Manning *et al*., 2008). Tuttle (2017) provides an interesting perspective on the underlying issues and potential abuses by the (private) medical profession, together with problems related to ascribing direct spill-overs of rabies from bats into humans (including that the final stages of rabies may only emerge many years after a long-forgotten bite from whatever source).

An alternative explanation for Constantine's original experiment with canids and other terrestrial mammals in a bat cave might be that they were infected by contaminated faeces or urine, a concern which has been expressed regarding other viruses (*e.g.* coronaviruses: Wacharapluesadee *et al.*, 2013). Evidence for lyssaviruses has been looked for and detected in the kidneys, bladder and/or gut in some other studies (although it is not clear whether this was in their associated innervation; see Section IV), as well as in the faeces in one (Allendorf *et al*., 2012), raising the possibility that excretory products might be a source of onward transmission of these as other viruses. This would be most likely in roosts: whilst bats take measures to ensure that they do not soil themselves when voiding their wastes,[111] 'subordinates' on lower perches may be exposed to pathogens when they clean themselves as a result of being the unwitting target of potentially contagious 'presents', as might bats flying below. However this would seem to be a long shot, based on a study of four naturally-infected dogs: this found that,

[110] Detected bites accounted for only seven of 41 reported fatal cases of humans as a result of bat rabies variants in the United States over the period 1990-2015; reported unprotected physical contact was associated with another ten, as well as being likely in a further seven (Dato *et al.*, 2016).

[111] http://boards.straightdope.com/sdmb/showthread.php?t=149867.

whilst rabies RNA could be detected in renal tissue and the uncentrifuged urine, no evidence for the virus itself was found (Sitprija *et al*., 2003).

Finally, Kuzmin *et al*. (2008c) reported the presence of infectious Lagos bat virus in the reproductive tract and in a vaginal swab of a single *Ei. helvum* which, they noted, suggests yet another potential route for onward transmission.

In between the initial establishment of any infection and its successful onward transmission to new hosts is the need for a virus to not only co-opt the existing host's cellular machinery to produce progeny but also to minimise the impact of its immune system. There is evidence that both the innate and acquired immune systems in those few species of bat which have been investigated have certain adaptive features which differ from those seen in the various other eutherian mammals which have been studied (reviewed by *e.g.* Schountz *et al.*, 2017). The ancient history of chiropterans and their apparently being the source of various viral groups which have subsequently spilled over to become established in other mammals have led to suggestions that the prolonged period of co-evolutionary arms races between the viruses and their hosts' immune systems means the bats are relatively unaffected by being infected.

Using the foregoing as a preliminary foundation, the following section will consider how lyssaviruses might have evolved during and after spill-overs from their presumed original arthropod hosts.

## 3. The Problem of the Origin of Rabies and Other Lyssaviruses

Lyssaviruses represent a monophyletic clade which is highly divergent from other rhabdovirid genera (Rupprecht *et al*., 2011). Apart from genomic studies, the fact that rabies virus may not have as severe an impact on bats as has been found to be the case for large terrestrial mammals has been suggested to be consistent with bats being the original natural reservoir for this and other lyssaviruses (Calisher *et al*., 2006; Rupprecht *et al*., 2011; Hayman *et al*., 2012c).

The apparent ancestry of the rhabdovirid family in arthropods (see Introduction) suggests that at least the progenitor of the genus *Lyssavirus* may have originated as a spill-over from these, whether passively through the consumption of infected arthropods or actively so by way of blood-sucking vectors. Thus Constantine (2009) suggested that transmission could be mediated

by the bites of mites and other haematophages; or even on the external surface of the larval and adult stages of dermestid beetles.[112]

Regardless of the transmission mechanism(s), it is generally assumed that a single past spill-over occurred, presumably in Eurasia based on current evidence. Whilst this seems the most parsimonious hypothesis, there might be the possibility that there have been multiple such events, including up to the present-day; that this could be the case is brought to mind by the *in vitro* studies on the Mokola virus, and possibly the lack of a known bat host for two other lyssaviruses (although inadequate sampling is just one possible alternative explanation).

The following will consider the two most likely routes for spilling over from arthropods, and how enzootic lineages might possibly have become established in bats.

***i. The Predatory Route*** A spill-over from eating infected arthropods could be through damage by the exoskeleton breaching the physicochemical barriers normally present in the lining of the oral or other portions of the digestive tract; or as a result of other means of uptake from the lumen. That this is conceivable is suggested by the efficacy of oral vaccination of present-day terrestrial mammals against rabies (see above). Such spill-overs would presumably be an ongoing series of one-off events, with no opportunity to spill back into their insect prey; the end result was that, by chance, the ancestral lyssavirus emerged with its panoply of genetic (pre-) adaptations to circulate within not only that particular host individual but also with the capacity to be shed in a manner which would allow its progeny to spread to others.

To this end, it is possible to conceive of a scenario where damage to the lining of the mouth might lead to uptake by afferent and efferent neurone nerve-endings (including those innervating the taste-buds) to establish a latent infection, reminiscent of that described above for α-herpesviruses; indeed there might be the possibility of a positive feedback (suggested above *vis-à-vis* the tongue, as a result of salivary viral excretions), including the additional potential for infection of the olfactory system from self-produced aerosols, whereby productive infection of one output leads to its spread to others in physical proximity.

An intriguing potential insight relates to the various origins have been proposed for the evolution of sanguivory in vampire bats, with five basic hypotheses having been advocated (Naish, 2007b). Of these, the most likely was considered to be that haematophagy evolved through the vampire bats' ancestors developing a taste for ectoparasitic arthropods

[112] Scavengers which feed on the integument (carpet beetles) as well as the underlying tissues of carcasses: https://en.wikipedia.org/wiki/Dermestidae.

congregating at wounds on terrestrial hosts. Noting that vampire bats are phyllostomids and developing on the ideas of Fenton (1992), Naish (2007c) proposed that their ancestors might have been part-time nectarivores which also fed on insects; the fact that they also fed on nectar would mean that they would be pre-adapted to lapping up blood from open wounds, whilst their phyllostomid dentition would favour the evolution of a more direct means for obtaining this alternative liquid diet through biting of the afflicted animal.[113] Thus, rather than getting infections from terrestrial mammals themselves, contagions could instead (or in addition) have been obtained from the associated ectoparasites, assuming that this was the route for the progenitor of a branch of a *Rabies virus* lineage to establish a foothold and ultimately give rise to the indigenous American clade.

Developing upon this, a variant is that grooming to be rid of body parasites (Marshall, 1982) may also be an alternative source of viruses from ectoparasites over and above the haematophagous route, with their ingestion being an alternative means to close the initial infective cycle. As considered in the following section, such grooming behaviour also opens up the possibility of infection from ingestion of recent salivary deposits from other bats infected with rabies-related or other viruses.

***ii. A 'Parasitic' Route*** The problem of how onward transmission might be achieved is avoided in the alternative, where the original arthropod host(s) and the potential new mammalian host(s) were (and conceivably still are) linked in a closed loop through the former transitioning from being the primary host to becoming a vector and then presumably ultimately becoming superfluous.[114] Intriguingly, it is noteworthy that Serra-Cobo *et al*. (2013) identified *European bat lyssavirus type 1* in blood clots from samples of various bat species in roosts in Spain, suggesting the potential for vector-borne infections even in the present-day.

The family Rhabdoviridae includes other members where haematophagous arthropods serve as vectors for viral transmission between mammalian hosts, including bats as well as humans: the so-called dimarhabdoviruses[115] in Africa and elsewhere (Aznar-Lopez *et al.*, 2013; Kading *et*

[113] Suggested to explain why no Old World haematophagous bats have evolved.

[114] It has been suggested that the hepatitis B virus may be transmitted by haematophagous insects (Blumberg, 1977; Echevarría and León, 2003; the latter authors also proposed that vampire bats may be intermediaries), as well as the hepatitis C flavivirus (presumably on the external mouthparts of such insects: Pybus *et al.*, 2007; Pybus and Thézé, 2016). Such a mechanism would explain how blood-borne hepaciviruses might circulate in species other than carnivorous ones.

[115] Dipteran-mammal-associated rhabdoviruses: an apparent phylogenetic 'supergroup' of arboviruses in this family (Kuzmin *et al*., 2009; Longdon *et al.*, 2015).

*al*., 2013; Binger *et al*., 2015; Li *et al*., 2015a, b);[116] and a group of insect-transmitted rhabdoviruses in bats from various parts of Southeast Asia.[117] Initial studies on the kotonkan virus from *Culicoides* midges in Nigeria (associated with a type of bovine ephemeral fever there) and the Obodhiang virus from mosquitoes (*Mansonia uniformis*) in Sudan found them to be related serologically to the Mokola virus and other lyssaviruses; they were thus proposed to be arboviruses[118] which bridged the gap separating lyssaviruses from their presumed arthropod ancestors (Shope, 1982; Calisher *et al*., 1989). Both viruses can be adapted to infect mammalian cell-lines through co-culture with 'helper' insect cells (Buckley, 1975);[119] they also were able to multiply efficiently in mouse neuroblastoma cells (Clark, 1980). However further studies have indicated that these two viruses should instead be assigned to the genus *Ephemerovirus*,[120] along with *Bovine ephemeral fever virus*, members of a group of Old World dimarhabdoviruses. Serological crossreactivity of the kotonkan and Obodhiang viruses with lyssaviruses is presumably due in part to the presence of a second glycoprotein ($G_{NS}$) more closely related in sequence to the single glycoprotein found in the last (Blasdell *et al*., 2012).

Given the relatively large exposed of area of the wings of bats and their hairlessness, these would be likely to be primary targets for haematophagous insects such as mosquitoes in roosts; other relatively large, more truly ectoparasitic arthropods (Madej *et al*., 2013)[121] would instead favour the shelter of hirsute parts of the torso, as they do in other mammals, or else are resident in the latters' roosts.[122,123] However, whilst such vectors may have played a bridging role in the

[116] Including evidence for a sustained infections by Kumasi rhabdovirus of the spleen in members (especially juveniles) in a colony of the fruit-bat *Ei. helvum* in West Africa, with serological evidence for transmission to pigs and humans living in the vicinity of the seasonal roost (Binger *et al*., 2015).

[117] No species were identified: http://www.healthmap.org/predict/

[118] A general term for **ar**thropod-**bo**rne viruses.

[119] *cf.* the Mokola virus, which was able to infect either mammalian or insect cell-lines.

[120] They have both since been recognised as distinct species: https://talk.ictvonline.org/taxonomy/ (2016 release).

[121] For a review of insect families, see Marshall (1982). Whilst most are blood-suckers, *Arixenia* spp. have biting mouthparts and are thought to be grazing commensals on the skin, its debris and exudates of a hairless molissid, whilst remaining in the host's roosts.

[122] Thus the likes of bed-bugs (*Cimex lectularius*) remain at the roost between intermittent blood-meals; interestingly, the latter infesting European bats may be closely related to those in humans (Marshall, 1982; Booth *et al.*, 2015; *cf.* Balvín *et al.*, 2012), and Williams *et al*. (1976; see also Adelman *et al.*, 2013) have noted possible ongoing spill-overs of a bunyavirid into guano-collectors from bed bugs in caves in Southeast Asia. If these or other ectoparasites can also infest *e.g.* canids, then this suggests a third alternative explanation for Constantine's (1967) finding that bat rabies may spill over into caged terrestrial mammals.

[123] Broadly speaking, the likes of female mosquitoes can be considered as ectoparasites, but the fact that they only transitorily 'parasitise' any one particular host individual means that they are generally categorised as 'micro-predators' (https://www.cdc.gov/parasites/about.html).

past, evidence for strong positive selection of known lyssaviruses would seem to argue against their continuing relevance, based on other studies on RNA viruses (Woelk and Holmes, 2002).

As noted above, the nature of the wings of bats means that there is a range of different possibilities regarding how a potentially competent virus may spill over, depending on the cell-types to which it is initially exposed by a biting arthropod (see Figure 2B).[124] Infections of the more distal portions (the dactylopatagium) would be most likely to infect cutaneous cells and, to a lesser extent, the associated sensory and autonomic innervation, given the relatively poor vascularisation of this wing segment. Infection of the former cells would lead to a local spread (presumably facilitated by viral variants which promote direct cell-to-cell transmission, as reported for the SHBRV and fixed strains of the rabies virus: Morimoto *et al*., 1996; Dietzschold *et al*., 2008); such a proposal would not seem unreasonable, given Capewell *et al.*'s (2016) having identified the dermis and subcutis as a reservoir for African trypanosomes in mice and humans.[125] On the other hand, infection of the sensory innervation (whether directly or through passive spread from epithelial cells: Figure 3) raises the possibility of establishing one 'reflex/reflux' route for the initiation of the (latent) infection of their neurone somata, recollecting the situation regarding human α-herpesvireses (see above).[126] In addition, direct or indirect infection of the autonomic innervation opens up possibilities regarding the spread of the virus centrally and thereafter to other output targets, such as the salivary glands, in a 'reflex arc'.

The plagiopatagium and uropatagium (as well as the much smaller protopatagium) are better vascularised in order to support the various muscles therein, and hence are likely to be less susceptible to infection as a result of a better-developed inflammatory response. Based on the hypothesis of Baer *et al.* (1990) and assuming that they are bestowed with a lesser density of nicotinic receptors than those muscles directly involved in the generation of the wing-beats themselves, they could represent a more long-term reserve for the initial 'smouldering'

[124] Whilst micro-predators such as mosquitoes might be expected to target capillaries, the fact that the (distal) wing acts as a respiratory surface in its own right for the emission of $CO_2$ may serve as a diversion for such vectors probing for blood-vessels.

[125] These authors found that this protozoan parasite, which normally circulates in the blood in mammals, was detectable in human skin biopsies as well as those of mice after intraperitoneal injection (without any evident inflammatory response, and in the absence of parasitaemia), with comparable results being seen when the latter were infected by bites from the natural vector, tsetse flies; moreover, these insects could be infected in turn by trypanosomes from skin preparations.

[126] Somewhat tangentially, this serves as one of the best means for diagnosis of presenting human patients *ante mortem* (Jackson, 2007); as well as being found in the muzzles of skunks and American red foxes (Balachandran and Charlton, 1994). It is tempting to speculate that this might reflect a 'primitive' viral adaptation.

development of an infection (see Section V.1) and thus a relatively prolonged latency for the development of an acute transmissive phase. Furthermore, their richer vascularisation increases the likelihood of an insect vector obtaining a blood-meal, as a potential route for their associated arboviruses to establish a more widespread infection such as is typical of other such viruses in terrestrial mammals. Related to this, Serra-Cobo *et al.* (2013) identified *European bat lyssavirus type 1* in blood clots from samples of various bat species in roosts in northern Spain; this might conceivably represent a relictual vector-mediated means of transmission as well as the evolution of an alternative, short-circuiting route. However there is the potential to infect the various other cell-types present, including the relatively exposed musculature and its attendant motor innervation, whilst probing for a blood-vessel.

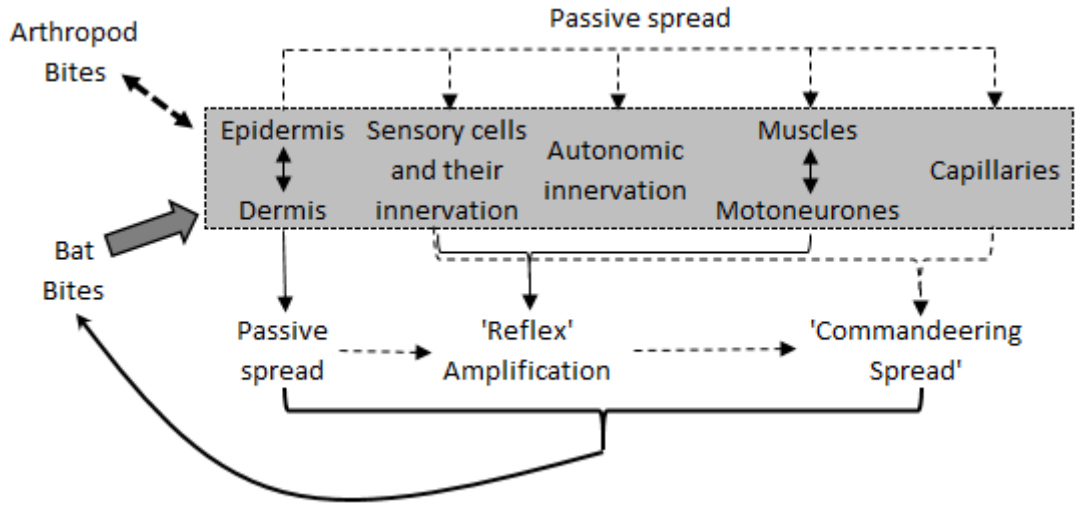



***Figure 3*** Various parallel conjoint routes are possible for the presumptive ancestral lyssavirus to become established in bats through an initial spill-over of an arbovirus from a haematophagous insect or other arthropod as a result of bites directed at various portions of the wings.

For the presumed ancestral lyssavirus to establish a 'wing-hold' in bats by taking advantage of the diversity of tissue-types which are accessible, it would need to be able to bind to the exposed domains of one or more types of molecule shared by these various types of cell. Studies on *Rabies virus* indicate that it is capable of infecting a large variety of cell-types *in vivo* and *in vitro* (Warrell, 2009). As noted in Section III.1, there is evidence that N-CAM plays a role as an acceptor for the initial infection by the rabies virus of striated muscles and motoneurones. Immunohistochemical studies in adult rats (Filiz *et al.*, 2002) have localised crossreactivity for N-CAM not only in various areas of the brain and spinal cord but also in the autonomic innervation of anterior portions of the digestive tract (see also Faure *et al.*, 2007), together with non-neuronal cells in its lining; and in the alveolar cells of the lung, the luminal membranes of

cells lining proximal convoluted tubules of the kidney, and the innervation of the male reproductive system. Little information would appear to be available regarding other tissues such as the salivary glands; however, given that N-CAM has been found to play a crucial role in cell division, migration and differentiation, it is likely to be at least transitorily expressed in various non-nervous tissues as a result of the normal turnover of cells therein, thereby presumably rendering them susceptible to rabies infection.

Thus, according to the hypothetical scenario summarised in Figure 3, the initial establishment of infection from haematophagous micro-predators or from grazers and other roost ectoparasites which targeted the wings of roosting bats could lead to multiple different types of cell serving as sites for viral replication for potential onward transmission to another biting insect host-vector. Presumably the infection of epithelial tissues would be the most likely to occur but passive spread could enhance the chances of other local constituent cell-types also being infected, including central afferent and efferent neurones (potentially as 'reflex/reflux' reservoirs for latent infection, with delayed amplification of viral production) and the autonomic system as a 'reflex arc' to further amplify viral spread, as well as through blood-borne infections (assuming that replication within the host can result in a sufficiently high viraemia for onward transmission to occur).

Moreover, as noted above, agonistic and other interactions between bats are likely to lead to bite-inflicted damage to their wings: for example, Shankar *et al*. (2004) reported that all *Ep. fuscus* caught to set up a captive colony had small, tooth-sized puncture scars on the wings when brought in from the field. This raises the possibility that contagion may be passed on through infection of the oral cavity (or digestive tract, if bits of the bitten bat's wing are swallowed) of the biting bat, helping to close the circulation cycle and render an insect vector redundant. The initial elements in this postulated scenario – infection of epithelial and neuronal cells – are reminiscent of the situation described above for the three herpes viruses; the main difference is that mucous epithelia are requisite for person-to-person contagion of the latter, something not necessary (or even likely) for insect-mediated transmission.

Whilst infections with herpes viruses are typically limited in their ability to spread within the central nervous system after reactivation in the vast majority of infected individuals, lyssaviruses must have further progressed to infect the rest of the central nervous system ('commandeering spread' in Figure 3), culminating in classical rabies-like syndromes with the virus effectively trying to take control of its own fate.

It is fully recognised that the above scenario, as it stands, represents a tentative 'Just So' story: to have any merit, it should generate testable hypotheses which, whilst not necessarily yielding direct supporting evidence, might narrow down the range of alternative possibilities. To recapitulate the foregoing, the underlying assumption is that lyssaviruses originated as spill-overs from haematophagous (or possibly grazing) arthropods directed mainly at the exposed wings of roosting bats; given the presumption that such spill-overs no longer occur, viral cycling is now enzootic within bats, being mainly mediated by bites in a bi-directional exchange of virions between the biter and the bitten. As indicated in Figure 2B, there are differences between regions of the wing in the number of tissues present. These comprise:

1) epithelial structures for the potential local spread of infection, such as seen with the SHBRV *in vitro*;
2) sensory elements for the potential (restricted?) central spread of infection, reminiscent of the likes of the α-herpesvirus model;
3) potential autonomic spread *via* the innervation of blood-vessels and other structures to other targets (including the salivary glands);
4) potential vascular spread, as suggested by experiments on mice with the SHBRV *in vivo*; and
5) potential musculo-neural spread in areas of the wing other than the dactylopatagium.

Probability-wise, infections would be most likely to occur in the order (1) to (5) based on relative areas of the tissue-types involved; the fact that it is mainly the dactylopatagium and the much smaller protpatagium which are exposed in roosting bats (Figure 1) further skews the probability (as with the original spill-overs from insects). A primary prediction would be that effective administration of virus to different components of the wing might lead to the establishment of local infection and, in some cases at least, 'reflex' amplification (Figure 3), with or without the development of an acquired (but not necessarily neutralising) immune response: Franka *et al*. (2008) found evidence for transient seroconversion after scratching of the protopatagium, but noted difficulties in standardising the dosage administered. The possibility of natural infection could be determined by sampling wings for evidence of lyssavirus infection, in particular in areas with existing tears and those which have healed: regenerated wing tissue is often unpigmented,[127] raising the possibility that it may serve as the focus for attacks by other bats and thus conceivably a means for promoting transmission of local infections to attacking bats. Moreover, the size and location of present or past damage may influence any infectivity: whilst bats can tolerate relatively large areas of damage to their

[127] Something which biologists take advantage of by punching holes as a means of identifying individuals over the long-term.

wings (Davis and Doster, 1972), that to particular portions can impair their manoeuverability in order to capture prey as well as to interact with other bats (Voigt, 2013). Recalling Constantine's (2009) suggestion that bats' susceptibility to rabies may be increased by stress, this may influence how an infected bat copes with the problem of infection (Pollock *et al.*, 2016), more especially in situations where there is increasing demand for energy intake as in the case of pregnant and lactating females (Ceballos-Vasquez *et al.*, 2015).

A major lacuna in this scenario is the 'leap' from the hypothetical insect-bat spill-over(s) to those where biting bats can act as vectors for transmission from those they have bitten. Implicit in the foregoing is that the evolving virus must be able to (i) infect oral or other tissues as a way of establishing a new infection, presumably by way of taste receptors or other cell-types in the buccal cavity being infected by wild-type viruses, with or without prior tissue damage; and also (ii) serve as a fount for the onward spread of contagion, either as a direct result or *via* prior infection of the autonomic nervous system at large (and thus *e.g.* the salivary glands) through the spread of virus in a 'reflex arc' from the presumed original focus in the wing.

Salivary secretion of virions has been presumed to be the default route for onward transmission in bats, based on the hypersalivation seen with classical rabies in terrestrial mammals. On the other hand, the apparent low level of virus shedding in the saliva of bats in many experiments raises questions about whether other routes may be implicated (reviewed by *e.g.* Vos et al., 2007; Freuling *et al*., 2009).[128] An alternative or additional possibility might be that lymphatic components of the bat's presumed homologue of Waldeyer's tonsillar ring could serve as a reservoir (*cf.* herpes virus 3, for example), as has been reported in the case of a dog which, nursed to recovery after infection with an apparently mild strain of wild rabies, continued to produce virus in its saliva (Fekadu *et al.*, 1983 [abstract]; see Section III.5.i).

Thus the 'classical model' – representing the acme of rabies-transmissability through the 'commandeering' of the nervous system at large in furious and paralytic forms of the disease – need not to apply as the standard aetiology for all clades within *Rabies virus*, at least in bats, never mind other members of the genus: comparable effects of the latter in terrestrial mammals may merely be the result of nascent paralogous effects reflecting their shared (bat) ancestry and the extent of the similarities of their genomes to that of the rabies virus itself, as exemplified

[128] However this presumes a marked hypersalivatory response; in the absence of the latter, it might be that stress from capture and sampling leads to a 'dryness of the mouth' in bats as in humans, at least; it presumably also leads to vocalisations and thus aerosol dispersion of the existing (virion-containing) oral fluids.

by the effects of the Lagos bat virus in mice (Badrane *et al.*, 2001; Markotter *et al.*, 2009). Hence, as noted in Section IV.1.i, there is little clear evidence for rabies-associated mortalities in wild populations. Where 'commandeering' infections do occur, there is evidence for variability in the onset of clinical disease in bats, as in terrestrial mammals (see Sections III.2 and 5): the record would seem to be for a *My. daubentonii* which was attacked by a cat and was kept in isolation after having one wing amputated but developed furious rabies nine months later due to a prior European bat lyssavirus type 2 infection (Pajamo *et al*., 2008); almost as long is one report for *Ep. fuscus* which was presumably infected in the wild with one of the species' variant viruses (Davis *et al.*, 2012b). This together with the lack of a fixed period between experimental infection and the death of affected bats suggests that, if a proportion of members of a cohort is infected, the variability in the onset of a terminal, potentially transmissive phase is spread out over time to maximise the chances of access to other, immunologically naïve individuals. This variability could be stochastic; or else in response to signals from the host regarding its current health-status: the latter would be reminiscent of the situation regarding arousal from latency noted above for α-herpesviruses, and recalls the ideas of Constantine (1967, 2009) that stress may play a role in determining the natural development of disease in infected wild populations of the migratory *T. b. mexicana*. Banyard *et al*. (2011) have noted that many experiments are terminated after a relatively restricted period (*e.g.* three months) so that this may give a false picture of the potential infectivity of a particular treatment; thus the ideal would be to have longer-term survival, with the possibility of including certain variables (including environmental temperature cycles and their effects on torpor as distinct from hibernation)[129] to determine whether the latter might have a modifying effect in triggering (or otherwise) the onset of clinical disease.

Regarding the other modes of infection – passive local spread and 'reflex' amplification – these might account for the often much greater prevalence of seropositive bats as compared with those which are evidently virus-positive. This disparity is generally assumed to be the result of failed infections, but the present scenario hypothesises that these may be effective in their own

[129] Thus, for example, resting vampire bats are essentially poikilothermic (reflecting their low-fat diet), at the opposite extreme from large pteropodids which are homoeothermic (Lyman, 1982; Geiser and Stawski, 2011). In tropical species, roosting torpor has been associated with 'shallow heterothermy', where a decrease in heart-rate leads to a slow down in the metabolic rate (O'Mara *et al.*, 2017a, b), with consequences for the immune system as well as any viral infection (*cf.* the fever hypothesis of O'Shea *et al.*, 2014b). Recalling of the results of Morimoto *et al.* (1996), Sadler and Enright (1959) found that whilst hibernation (4 ºC) arrested the development of experimental rabies infection in *Antrozous pallidus*, induced daily torpor (22 *vs.* 37 ºC) had little apparent effect.

right with the potential for onward transmission serving as the necessary intermediate 'missing link'in the evolution of pathogenesis which culminates in classical rabies such as seen in canids today. Moreover, were 'reflex' amplification to apply, then infected bats might represent latent carriers as in the three herpes viruses, thereby rendering models such as those of Dimitrov *et al.* potentially relevant (see Section IV.3.i). The often high proportion of seropositivity would seem to suggest that the original infection occurs in relatively young bats, after any pre-existing maternally-derived immunity has worn off (Scott and Nel, 2016). Furthermore, the presumably limited exposure of many wing epithelial cells to blood-borne antibodies might mean that, if infected, islands of these can persist as a result of their ability to down-regulate local innate immune responses: they thus might represent an additional category of immunologically-privileged tissues as a reservoir for potential further spread within the individual (somewhat reminiscent of the proposed role of muscles in classical rabies infections) as well as for onward transmission to others.

## VI. Conclusions

Most viruses which infect the nervous system at large are 'opportunistic': their spread is self-defeating, in that they lead to the the demise of the present host without further ensuring onward transmission to new hosts (Koyuncu *et al.*, 2013; Swanson and McGavern, 2015). Notable exceptions include human herpes viruses 1-3, which normally establish latent infections limited to particular sensory neurones with the potential for re-emergence to infect new hosts; however, as with many other viruses, the unconstrained central spread of these in certain individuals leads to self-defeating neuro-inflammatory disease.

The apotheosis of such passive strategies is the 'furious' rabies condition, where infection most effectively commandeers the present host's behaviour and viral output in order to try to ensure a new host at the expense of the current one – the latter represents a disposable soma, rather than a potentially threatened one as typical of the inherent 'reflex' responses of herpes viral infections. Such emergent specialisation reflects in part an increasing sophistication in the virus' ability to subvert its host's immune defence systems. On the other hand, there is evidence that the rabies virus in bats may not always be associated with the classical furious or paralytic symptoms seen in many terrestrial mammals, although it is debatable whether this reflects abortive infections which failed to establish a sustainable route for onward transmission.

Considered in this context, *Rabies virus* is a "riddle wrapped in an enigma."[130] The riddle is how strains circulate within various species of bats in comparison to their epidemiology in various 'typical' terrestrial mammalian populations; and how this relates to that of other lyssaviruses in their presumed natural reservoirs. The enigma is the nature of the presumed transitional stages in the evolution of the genus *Lyssavirus* from an arthropod virus *via* being able to not only infect and presumably become enzootic in mammals (presumably in bats initially) but also to thereafter to ultimately acquire a sophisticated strategy where, rather than being dependent on the host's survival (including being sensitive to signals which may presage the latter's demise, as exemplified by α-herpesviruses), the virus instead seeks to take control of its own future through subverting its current host's nervous system at large and potential output sources for its own onward propagation.

Considering the enigma first (since this might provide clues to answering the inherent riddle), it is hypothesised that the wings of bats not only represent a unique adaptation for powered flight but that they thereby also present a large surface area for the attention of haematophagous arthropods when roosting. Based on what is known about the properties of various strains of *Rabies virus*, in particular, various stages have been proposed as to how the classic furious and paralytic manifestations of infections might have arisen (Figure 3). Moreover, these intermediate stages may still prevail in bats (whether as acute, chronic or 'smouldering' infections), thereby suggesting experiments which might help to resolve the riddle of the occurrence of rabies *vs.* the resulting seroprevalence against it in bats.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## References[132]


Adelman, Z. N., Miller, D. M., and Myles, K. M. (2013) Bed Bugs and Infectious Disease: A Case for the Arboviruses. *PLoS Pathog.* **9**, e1003462.


[130] https://idioms.thefreedictionary.com/a+riddle+wrapped+in+an+enigma; it is a contraction of a foreign policy statement by Churchill in 1939 with regard to the USSR, which concluded that the key to resolving the issue was in understanding their national interests. Whilst the latter reflected group selection, the phrase as used here relates to natural selection at the level the genes in lyssaviruses and how the 'interests' of individual virions have evolved over time in order to produce a sustainable infection of existing and thenceforth potential new host reservoirs.

[131] To an erstwhile neurobiologist with an acquired allergy to things immunological.

[132] The content of all webpage references was confirmed 3/i/2018

Afshar, A. (1979) A review of non-bite transmission of rabies virus infection. *Brit. Vet. J.* **135**, 142-148.

Aghomo, H.O., and Rupprecht, C.E. (1990) Further studies on rabies virus isolated from healthy dogs in Nigeria. *Vet. Microbiol*. **22**, 17-22.

Aguilar-Setien, A., Loza-Rubio, E., Salas-Rojas, M., Brisseau, N., Cliquet, F., *et al*. (2005) Salivary excretion of rabies virus by healthy vampire bats. *Epidemiol. Infect*. **133**, 517-522.

Aitken, T.H., Kowalski, R.W., Beaty, B.J., Buckley, S.M., Wright, J.D., *et al.* (1984) Arthropod studies with rabies-related Mokola virus. *Amer. J. Trop. Med. Hyg.* **33**, 945-952.

Aliyu, T.B., De, N., Yenda, E.N., and Lynn, M. (2010) Prevalence of rabies virus antigens in apparently healthy dogs in Yola, Nigeria. *Researcher* **2**, 1-14.

Allendorf, S.D., Cortez, A., Heinemann, M.B., Harary, C.M.A., Antunes, J.M.A., *et al*. (2012) Rabies virus distribution in tissues and molecular characterization of strains from naturally infected non-hematophagous bats. *Virus Res.* **165**, 119-125.

Almeida, M. F., Martorelli, L. F., Aires, C. C., Sallum, P. C., Durigon, E. L., *et al*. (2005) Experimental rabies infection in haematophagous bats *Desmodus rotundus*. *Epidemiol. Infect*. **133**, 523-527.

Amarasinghe, G.K., Bào, Y., Basler, C.F., Bavari, S., Beer, M., *et al*. (2017) Taxonomy of the order Mononegavirales: Update 2017. *Arch. Virol.* 1-12.

Amaro, Y. (2016) Villagers 'unworried' by animal-borne diseases. *Phnom Penh Post* http://www.phnompenhpost.com/national/villagers-unworried-animal-borne-diseases.

Amengual, B., Bourhy, H., López-Roig, M., and Serra-Cobo, J. (2007) Temporal dynamics of European bat Lyssavirus type 1 and survival of *Myotis myotis* bats in natural colonies. *PLoS One* **2**, 566.

Araujo, D.B., Martorelli, L.A., Kataoka, A.P.G., Campos, A.C., Rodrigues, C.S., *et al.* (2014) Antibodies to rabies virus in terrestrial wild mammals in native rainforest on the north coast of São Paulo State, Brazil. *J. Wildl. Dis.* **50**, 469-477.

Arita, H.T., Vargas-Barón, J., and Villalobos, F. (2014) Latitudinal gradients of genus richness and endemism and the diversification of New World bats. *Ecography* **37**, 1024-1033.

Aznar-Lopez, C., Vazquez-Moron, S., Marston, D.A., Juste, J., Ibanez, C., *et al.* (2013) Detection of Rhabdovirus Viral RNA in Oropharyngeal Swabs and Ectoparasites of Spanish Bats. *J. Gen. Virol.* **94**, 69-75.

Baby, J., Mani, R.S., Abraham, S.S., Thankappan, A.T., Pillai, P.M., *et al*. (2015) Natural Rabies Infection in a Domestic Fowl (*Gallus domesticus*): A Report from India. *PLoS Negl. Trop. Dis.* **9**, e0003942.

Badrane, H., and Tordo, N. (2001) Host switching in Lyssavirus history from the Chiroptera to the Carnivora orders. *J. Virol.* **75**, 8096-8104.

Badrane, H., Bahloul, C., Perrin, and Tordo, N. (2001) Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. *J. Virol.* **75**, 3268-3276.

Baer, G.M. (2007) The History of Rabies. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press, pp. 1-22.

Baer, G.M., and Bales, G.L. (1967) Experimental rabies infection in the Mexican freetail bat. *J. Infect. Dis.* **16**, 82-90.

Baer, G.M., and Smith, J.S. (1991) Rabies in nonhematophagous bats. *In* The Natural History of Rabies, (G. M. Baer, ed.), CRC Press, Boca Raton, FL, USA, 341-366.

Baer, G.M., Shaddock, J.H., Quirion, R., Dam, T.V. and Lentz, T.L. (1990) Rabies susceptibility and acetylcholine receptor. *Lancet* **335**, 664-665.

Baker, K.S., Suu-Ire, R., Barr, J., Hayman, D.T., Broder, C.C., *et al.* (2014) Viral antibody dynamics in a chiropteran host. *J. Anim. Ecol.* **83**, 415-428.

Baker, M. L., Schountz, T., and Wang, L.F. (2012) Antiviral Immune Responses of Bats: A Review. *Zoon. Publ. Health*

Balachandran, A., and Charlton, K. (1994) Experimental rabies infection of non-nervous tissues in skunks (*Mephitis mephitis*) and foxes (*Vulpes vulpes*). *Vet. Pathol.* **31**, 93-102.

Balvín, O., Munclinger, P., Kratochvíl, L., and Vilímová, J. (2012) Mitochondrial DNA and morphology show independent evolutionary histories of bedbug *Cimex lectularius* (Heteroptera: Cimicidae) on bats and humans. *Parasitol. Res.* **111**, 457-469.

Banyard, A. C., Hayman, D., Johnson, N., McElhinney, L., and Fooks, A.R. (2011) Bats and Lyssaviruses. *Adv. Vir. Res.* **79**, doi:10.1016/B978-0-12-387040-7.00012-3.

Banyard, A.C., and Fooks, A.R. (2017) The impact of novel lyssavirus discovery. *Microbiol. Aust*. **38**, 18-21.

Banyard, A.C., Evans, J.S., Luo, T.R., and Fooks, A.R. (2014a) Lyssaviruses and bats: emergence and zoonotic threat. *Viruses* **6**, 2974-2990.

Banyard, A.C., Healy, D.M., Brookes, S.M., Voller, K., Hicks, D.J., *et al.* (2014b) Lyssavirus infection: 'Low dose, multiple exposure' in the mouse model. *Vir. Res.* **181**, 35-42.

Barrett, J. (2011) Lyssaviruses.*In* S.H. Newman, H.E. Field, C.E. de Jong and J.H. Epstein (eds.) *Investigating the role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests.* Rome: Food and Agriculture Organization of the United Nations.

Begon, M. (2009) Ecological epidemiology. *In* S.A. Levin, S.R. Carpenter, H.C.J. Godfray, A.P. Kinzig, M. Loreau *et al*. (eds.), *The Princeton Guide to Ecology*. Princeton University Press, pp. 220-226.

Begon, M., Townsend, C.R., and Harper, J.L. (2006) *Ecology: from individuals to ecosystems*. 4th edn. Malden: Blackwell Publishing.

Bell, J.F., and Moore, G.J. (1971) Susceptibility of Carnivora to rabies virus administered orally. *Amer. J. Epidemiol.* **93**, 176-182. [Abstract]

Belshaw, R., Gardner, A., Rambaut, A., and Pybus, O.G. (2008) Pacing a small cage: mutation and RNA viruses. *Trends Ecol. Evol.* **23**, 188-193.

Berentsen, A.R., Dunbar, M.R., Becker, M.S., M'soka, J., Droge, E., *et al*. (2013) Rabies, canine distemper, and canine parvovirus exposure in large carnivore communities from two Zambian ecosystems. *Vect.-Borne Zoon. Dis.* **13**, 643-649.

Binger, T., Annan, A., Drexler, J.F., Muller, M.A., Kallies, R., *et al.* (2015) A novel rhabdovirus isolated from the straw-coloured fruit bat *Eidolon helvum* with signs of antibodies in swine and humans. *J. Virol*. **89**, 4588-4597.

Bingham, J. (2005) Canine rabies ecology in southern Africa. *Emerg. Infect. Dis*. **11**, 1337-1342.

Blackwood, J.C., Streicker, D.G., Altizer, S., and Rohani, P. (2013a) Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proc. Nat. Acad. Sci. USA* **110**, 20837-20842.

Blackwood, J.C., Streicker, D.G., Altizer, S., and Rohani, P. (2013b) Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. Supporting Online Material. *Proc. Nat. Acad. Sci. USA* **110**, 20837-20842.

Blancou, J. (2004) Rabies in Europe and the Mediterranean Basin: from antiquity to the 19th century. *In* A.A. King, A.R. Fooks, M. Aubert and A.I. Wandeler (eds.), *Historical perspective of Rabies in Europe and the Mediterranean Basin*. Paris, France: OIE. pp. 15-24.

Blasdell, K.R., Voysey, R., Bulach, D., Joubert, D.A., Tesh, R.B., *et al*. (2012) Kotonkan and Obodhiang viruses: African ephemeroviruses with large and complex genomes. *Virology* **425**, 143-153.

Bloom, D.C., and Dhummakupt, A. (2016) The Herpes Simplex Viruses. *In* C.S. Reiss (ed.), *Neurotropic Viral Infections*. *Volume 2: Neurotropic Retroviruses, DNA Viruses, Immunity and Transmission*. Springer, pp. 111-134.

Blumberg, B.S. (1977) Australia antigen and the biology of hepatitis B. *Science* **197**, 17-25.

Bonaccorso, F.J., and McGuire, L.P. (2013) Modeling the colonization of Hawaii by hoary bats (*Lasiurus cinereus*). *In* R. Adams and S.C. Pedersen (eds.), *Bat evolution, ecology, and conservation*. New York: Springer, pp. 187-205.

Booth, W., Balvín, O., Vargo, E.L., Vilímová, J., and Schal, C. (2015) Host association drives genetic divergence in the bed bug, *Cimex lectularius*. *Mol. Ecol.* **24**, 980-992.

Borucki, M.K., Chen-Harris, H., Lao, V., Vanier, G., Wadford, D.A., *et al.* (2013) Ultra-deep sequencing of intra-host rabies virus populations during cross-species transmission. *PLoS Negl. Trop. Dis*. **7**, e2555.

Bourhy, H., Kissi, B., Audry, L., Smreczak, M., Sadkowska-Todys, M., *et al.* (1999) Ecology and evolution of rabies virus in Europe. *J. Gen. Virol.* **80**, 2545-2557.

Bourhy, H., Kissi, B., Audry, L., Smreczak, M., Sadkowska-Todys, M., *et al.* (1999) Ecology and evolution of rabies virus in Europe. *J. Gen. Virol*. **80**, 2545-2557.

Bourhy, H., Reynes, J.M., Dunham, E.J., Dacheux, L., Larrous, F., *et al*. (2008) The origin and phylogeography of dog rabies virus. *J. Gen. Virol.* **89**, 2673-2681.

Bowen, R.A., O'Shea, T.J., Shankar, V., Neubaum, M.A., Neubaum, D.J., *et al*. (2013) Prevalence of neutralizing antibodies to rabies virus in serum of seven species of insectivorous bats from Colorado and New Mexico. *J. Wildl. Dis*. **49**, 367-374.

Brunker, K., Hampson, K., Horton, D.L., and Biek, R. (2012) Integrating the landscape epidemiology and genetics of RNA viruses: rabies in domestic dogs as a model. *Parasitology* **139**, 1899-1913.

Buckley, S.M. (1975) Arbovirus infection of vertebrate and insect cell cultures, with special emphasis on Mokola, Obodhiang, and Kotonkan viruses of the rabies serogroup. *Anns. N. Y. Acad. Sci*. **266**, 241-250.

Bullen, R. and McKenzie, N.L. (2001) Bat airframe design: flight performance, stability and control in relation to foraging ecology. *Aust. J. Zool*. **49**, 235-261.

Califf, K.J., Ratzloff, E.K., Wagner, A.P., Holekamp, K.E. and Williams, B.L., 2013. Forces shaping major histocompatibility complex evolution in two hyena species. *J. Mammal.* **94**, 282-294.

Calisher, C.H., Karabatsos, N., Zeller, H., Digoutte, J.-P., Tesh, R.B., *et al*. (1989) Antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods. *Intervirology* **30**, 241-257.

Capewell, P., Cren-Travaillé, C., Marchesi, F., Johnston, P., Clucas, C., *et al.* (2016) The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *eLife* **5**, e17716.

Carneiro, A.J.B., Franke, C.R., Stöcker, A., dos Santos, F., Úngar de Sá, J.E., *et al*. (2010) Rabies Virus RNA in Naturally Infected Vampire Bats, Northeastern Brazil. *Emerg. Infect. Dis.* **16**, 2004-2006.

Castilho, J.G., Carnieli, P., Oliveira, R.N., Fahl, W.O., Cavalcante, R., *et al*. (2010) A comparative study of rabies virus isolates from hematophagous bats in Brazil. *J. Wildl. Dis.* **46**, 1335-1339.

Ceballos, N.A., Morón, S.V., Berciano, J.M., Nicolás, O., López, C.A., *et al*. (2013) Novel lyssavirus in bat, Spain. *Emerg. Infect. Dis*. **19**, 793-795.

Ceballos-Vasquez, A., Caldwell, J.R., and Faure, P.A. (2015) Seasonal and reproductive effects on wound healing in the flight membranes of captive big brown bats. *Biol. Open* **4**, 95-103.

Charlton, K.M., Casey, G.A., and Campbell, J.B. (1983) Experimental rabies in skunks: mechanisms of infection of the salivary glands. *Can. J. Comp. Med.* **47**, 363-369.

Cheney, J.A. (2015) *Flying with membrane wings: how bats shape their wings with their hindlimbs, skin mechanics, and wing membrane muscles*. Brown University: Ph.D. Thesis.

Cheney, J.A., Konow, N., Middleton, K.M., Breuer, K.S., Roberts, T.J., *et al*. (2014) Membrane muscle function in the compliant wings of bats. *Bioinsp. Biomim.* **9**, 025007.

Childs, J.E., and Real, L.A. (2007) Epidemiology. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press.

Chiou, H.Y., Hsieh, C.H., Jeng, C.R., Chan, F.T., Wang, H.Y., *et al.* (2014) Molecular characterization of cryptically circulating rabies virus from ferret badgers, Taiwan. *Emerg. Infect. Dis*. **20**, 790-798.

Clark, H.F. (1980) Rabies serogroup viruses in neuroblastoma cells: propagation,"autointerference," and apparently random back-mutation of attenuated viruses to the virulent state. *Infect. Immun.* **27**, 1012-1022.

Cleaveland, S., and Dye, C. (1995) Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasitology* **111**, S33-S47.

Cleaveland, S., Packer, C., Hampson, K., Kaare, M., Kock, R., *et al*. (2008) The multiple roles of infectious diseases in the Serengeti ecosystem. *In* A.R.E. Sinclair, C. Packer, S.A. Mduma, and J.M. Fryxell (eds.), *Serengeti III: Human Impacts on Ecosystem Dynamics*. Chicago: Chicago Univ. Press, pp.209-239.

Coertse, J., Markotter, W., le Roux, K., Stewart, D., Sabeta, C.T. , *et al.* (2017) New isolations of the rabies-related Mokola virus from South Africa. *BMC Vet. Res.* **13**, 37.

Condori-Condori, R.E., Streicker, D.G., Cabezas-Sanchez, C., and Velasco-Villa, A. (2013) Enzootic and epizootic rabies associated with vampire bats, Peru. *Emerg. Infect. Dis*. **19**, 1463-1469.

Conomy, J.P., Leibovitz, A., McCombs, W., and Stinson, J. (1977) Air-borne rabies encephalitis: demonstration of rabies virus in the human central nervous system. *Neurology* **27**, 67-69.

Constantine, D.G. (1967) Bat rabies in the southwestern United States. *Pub. Health Repts.* **82**, 867-888.

Constantine, D.G. (1986) Absence of prenatal infection of bats with rabies virus. *J. Wildl. Dis*. **22**, 249 -250.

Constantine, D.G. (2009) Bat rabies and other lyssavirus infections. Reston, Va., U.S. Geological Survey Circular 1329, 68 pp.

Constantine, D.G., Emmons, R.W., and Woodie, J.D. (1972) Rabies virus in nasal mucosa of naturally infected bats. *Science* **175**, 1255-1256.

Coulon, P., Ternaux, J.P., Flamand, A. and Tuffereau, C. (1998) An avirulent mutant of rabies virus is unable to infect motoneurons *in vivo* and *in vitro*. *J. Virol*. **72**, 273-278.

Courts, S.E. (1998) Dietary strategies of Old World fruit bats (Megachiroptera, Pteropodidae): how do they obtain sufficient protein? *Mamm. Rev.* **28**, 185-194.

Covault, J., and Sanes, J.R. (1986) Distribution of N-CAM in synaptic and extrasynaptic portions of developing and adult skeletal muscle. *J. Cell Biol.* **102**, 716-730.

Crichton, E.G., and Krutzsch, P.H., eds. (2000) *Reproductive biology of bats*. Academic Press.

Crowley, G.V., and Hall, L.S. (1994) Histological observations on the wing of the grey-headed flying-fox (*Pteropus poliocephalus*) (Chiroptera, Pteropodidae). *Aust. J. Zool.* **42**, 215-231.

Dato, V.M., Campagnolo, E.R., Long, J., and Rupprecht, C.E. (2016) A systematic review of human bat rabies virus variant cases: evaluating unprotected physical contact with claws and teeth in support of accurate risk assessments. *PLoS One* **11**, e0159443.

Davis, A.D., Dupuis, M., and Rudd, R.J. (2012b) Extended incubation period of rabies virus in a captive big brown bat (*Eptesicus fuscus*). *J. Wildl. Dis*. **48**, 508-511.

Davis, A.D., Gordy, P., and Bowen, R.A. (2013b) Unique Characteristics of Bat Rabies viruses in Big Brown Bats (*Eptesicus fuscus*). *Arch. Virol*. **158**, 809-820.

Davis, A.D., Gordy, P., Rudd, R., Jarvis, J.A., and Bowen, R.A. (2012a) Naturally acquired rabies virus infections in wild-caught bats. *Vect.-Borne Zoon. Dis.* **12**, 55-60.

Davis, A.D., Jarvis, J.A., Pouliott, C., and Rudd, R.J. (2013c) Rabies virus infection in *Eptesicus fuscus* bats born in captivity (naïve bats). *PloS One* **8**, e64808.

Davis, A.D., Jarvis, J.A., Pouliott, C.E., Shannon, M.D., and Rudd, R.J. (2013a) Susceptibility and pathogenesis of little brown bats (*Myotis lucifugus*) to heterologous and homologous rabies viruses. *J. Virol*. **87**, 9008-9015.

Davis, A.D., Rudd, R.J., and Bowen, R.A. (2007) Effects of aerosolized rabies virus exposure on bats and mice. *J. Infect. Dis.* **195**, 1144-1150.

Davis, P.L., Bourhy, H., and Holmes, E.C. (2006) The evolutionary history and dynamics of bat rabies virus. *Infect. Genet. Evol*. **6**, 464-473.

Davis, R,. and Doster, S. (1972). Wing repair in pallid bats. *J. Mammal*. **53**, 377-378.

de Thoisy, B., Bourhy, H., Delaval, M., Pontier, D., Dacheux, L., *et al.* (2016) Bioecological drivers of rabies virus circulation in a neotropical bat community. *PLoS Negl. Trop. Dis.* **10**, e0004378.

Dechmann, D.K., and Safi, K. (2005) Studying communication in bats. *Cogn. Brain Behav.* **9**, 479-496.

Dietzgen, R.G., Calisher, C.H., Kurath, G., Kuzmin, I.V., Rodriguez, L.L., *et al.* (2012) Rhabdoviridae. *In* A.M.Q. King M.J. Adams, E.B. Carstens and E.J. Lefkowitz (eds.) *Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses*. Amsterdam: Elsevier, pp. 684-713.

Dietzschold, B., Li, J., Faber, M., and Schnell, M. (2008) Concepts in the pathogenesis of rabies. *Future Virol*. **3**, 481-490.

Dietzschold, B., Wiktor, T.J., Trojanowski, J.Q., Macfarlan, R.I., Wunner, W.H., *et al*. (1985). Differences in cell-to-cell spread of pathogenic and apathogenic rabies virus *in vivo* and *in vitro. J. Virol*. **56**, 12-18.

Dimitrov, D.T. and Hallam, T.G. (2009) Effects of immune system diversity and physical variation of immunotypic mixing on the dynamics of rabies in bats. *J. Biol. Dynam*.

Dimitrov, D.T., Hallam, T.G., Rupprecht, C.E., and McCracken, G.F. (2008) Adaptive modeling of viral diseases in bats with a focus on rabies. *J. Theor. Biol*. **255**, 69-80.

Dimitrov, D.T., Hallam, T.G., Rupprecht, C.E., Turmelle, A.S., and McCracken, G.F. (2007) Integrative models of bat rabies immunology, epizootiology and disease demography. *J. Theor. Biol.* **245**, 498-509.

Dobson, A., and Carper, E.R. (1996) Infectious diseases and human population history. *Amer. Inst. Biosci.* **46**, 115-126.

Dzikwi, A.A., Kuzmin, I.I., Umoh, J.U., Kwaga, J.K., Ahmad, A.A., *et al*. (2010) Evidence of Lagos bat virus circulation among Nigerian fruit bats. *J. Wildl. Dis.* **46**, 267-271.

Dzikwi, A.A., Umoh, J.U., and Kwaga, J.K. (2017) Phylogenetic Analysis of Rabies Virus Isolates from Dogs in Plateau State, Nigeria. *J. Biotech. Res.* **3**, 10-14.

East, M.L., Hofer, H., Cox, J.H., Wulle, U., Wiik, H., *et al*. (2001) Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas. *Proc. Nat. Acad. Sci. USA* **98**, 15026-15031.

Echevarría, J.M., and León, P. (2003) Epidemiology of viruses causing chronic hepatitis among populations from the Amazon Basin and related ecosystems. *Cadernos de saúde pública* **19**, 1583-1591.

Eggerbauer, E., Pfaff, F., Finke, S., Höper, D., Beer, M., *et al*. (2017) Comparative analysis of European bat lyssavirus 1 pathogenicity in the mouse model. *PLoS Negl. Trop. Dis.* **11**, e0005668.

Eick, G.N., Jacobs, D.S., and Matthee, C.A. (2005) A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Mol. Biol. Evol.* **22**, 1869-1886.

Escobar, L., Peterson, A.T., Favi, M., Yung, V., and Medina-Vogel, G. (2015) Bat-borne rabies in Latin America. *Rev. Inst. Med. Trop. Sao Paulo* **57**, 63-72.

Eshleman, E., Shahzad, A., and Cohrs, R.J. (2011) Varicella zoster virus latency. *Future Virol.* **6**, 341-355.

Everard, C.O., and Everard, J.D. (1988) Mongoose rabies. *Revs. Infect. Dis*. **10**, S610-S614.

Everard, C.O., and Everard, J.D. (1992) Mongoose rabies in the Caribbean. *Anns. N. Y. Acad. Sci*. **653**, 356-366.

Everard, C.O., Baer, G.M., Alls, M.E., and Moore, S.A. (1981) Rabies serum neutralizing antibody in mongooses from Grenada. *Trans. Roy. Soc. Trop. Med. Hyg*. **75**, 654-666.

Everard, C.O., Murray, D., and Gilbert, P.K. (1972) Rabies in Grenada. *Trans. Roy. Soc. Trop. Med. Hyg.* **66**, 878-888.

Everard, C.O.R., Baer, G.M., and James, A. (1974) Epidemiology of mongoose rabies in Grenada. *J. Wildl. Dis.* **10**, 190-196.

Faber, M., Faber, M.L., Papaneri, A., Bette, M., Weihe, E., *et al.* (2005) A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. *J. Virol.* **79**, 14141-14148.

Faure, C., Chalazonitis, A., Rhéaume, C., Bouchard, G., Sampathkumar, S., *et al.* (2007) Gangliogenesis in the enteric nervous system: Roles of the polysialylation of the neural cell adhesion molecule and its regulation by bone morphogenetic protein-4. *Dev. Dynam.* **236**, 44-59.

Faure, P.A., Re, D.E., and Clare, E.L. (2009) Wound healing in the flight membranes of big brown bats. *J. Mammal.* **90**, 1148-1156.

Feder, H.M., Nelson, R., and Reiher, H.W. (1997) Bat bite? *Lancet* **350**, 1300.

Fekadu, M. (1991) Latency and aborted rabies. *In* G.M. Baer (ed.), *The Natural History of Rabies* (2nd edition). Boca Raton: CRC Press, pp. 191-198.

Fekadu, M., Shaddock, J.H., and Baer, G.M. (1981) Intermittent excretion of rabies virus in the saliva of a dog two and six months after it had recovered from experimental rabies. *Amer. J. Trop. Med. Hyg.* **30**, 1113-1115 [Abstract].

Fekadu, M., Shaddock, J.H., Chandler, F.W., and Baer, G.M. (1983) Rabies virus in the tonsils of a carrier dog. *Arch. Virol.* **78**, 37-47 [Abstract].

Fenton, M.B. (1992) Wounds and the origin of blood-feeding in bats. *Biol. J. Linn. Soc.* **47**, 161-171.

Fenton, M.B. (2003) Eavesdropping on the echolocation and social calls of bats. *Mammal Rev*. **33**, 193-204.

Filiz, S., Dalcik, H., Yardimoglu, M., Gonca, S., and Ceylan, S. (2002) Localization of neural cell adhesion molecule (N-CAM) immunoreactivity in adult rat tissues. *Biotech. Histochem.* **77**, 127-135.

Fornes, A., Lord, R. D., Kuns, M. L., Larghi, O. P., Fuenzalida, E., *et al.* (1974) Control of bovine rabies through vampire bat control. *J. Wildl. Dis*. **10**, 310-316.

Franka, R., Johnson, N., Müller, T., Vos, A., Neubert, L., *et al*. (2008) Susceptibility of North American big brown bats (*Eptesicus fuscus*) to infection with European bat lyssavirus type 1. *J. Gen. Virol*. **89**, 1998-2010.

Frantz, L.A., Mullin, V.E., Pionnier-Capitan, M., Lebrasseur, O., Perri, A., *et al*. (2016) Genomic and archaeological evidence suggests a dual origin of domestic dogs. *Science* **352**, 1228-1231.

Freuling, C., Vos, A., Johnson, N., Kaipf, I., Denzinger, A., *et al*. (2009) Experimental infection of serotine bats (*Eptesicus serotinus*) with European bat lyssavirus type 1a. *J. Gen. Virol*. **90**, 2493-2502.

Freuling, C.M., Binger, T., Beer, M., Adu-Sarkodie, Y., Schatz, J., *et al*. (2015) Lagos bat virus transmission in an *Eidolon helvum* bat colony, Ghana. *Virus Res*. **210**, 42-45.

Garnett, C.T., Talekar, G., Mahr, J.A., Huang, W., Zhang, Y., *et al.* (2009) Latent Species C Adenoviruses in Human Tonsil Tissues. *J. Virol.* **83**, 2417-2428.

Geiser, F., and Stawski, C. (2011) Hibernation and torpor in tropical and subtropical bats in relation to energetics, extinctions, and the evolution of endothermy. *Integ. Compar. Biol.* **51**, 337-348.

George, D.B., Webb, C.T., Farnsworth, M.L. O'Shea, T.J., Bowen, R.A., *et al*. (2011) Host and viral ecology determine bat rabies seasonality and maintenance. *Proc. Nat. Acad. Sci. USA* **108**, 10208-10213.

Gibbons, R.V. (2002) Cryptogenic rabies, bats, and the question of aerosol transmission. *Anns. Emergency Med.* **39**, 528-536.

Gilbert, A.T., Petersen, B.W., Recuenco, S., Niezgoda, M., Gómez, J., *et al*. (2012) Evidence of Rabies Virus Exposure among Humans in the Peruvian Amazon. *Amer. J. Trop. Med. Hyg.* **87**, 206-215.

Goldsmith, E.W., Renshaw, B., Clement, C.J., Himschoot, E.A., Hundertmark, K.J. *et al.* (2016) Population structure of two rabies hosts relative to the known distribution of rabies virus variants in Alaska. *Mol. Ecol*. **25**, 675-688.

Griffin, D.E. (2003) Immune responses to RNA-virus infections of the CNS. *Nature Revs. Immunol.* **3**, 493-502.

Grimm, D. (2015) Dawn of the dog. *Science* **348**, 274-279.

Gunawardena, P.S., Marston, D.A., Ellis, R.J., Wise, E.L., Karawita, A.C., *et al*. (2016) Lyssavirus in Indian Flying Foxes, Sri Lanka. *Emerg. Infect. Dis.* **22**, 1456-1459.

Hamir, A.N., Moser, G., and Rupprecht, C.E. (1996) Clinicopathologic variation in raccoons infected with different street rabies virus isolates. *J. Vet. Diagn. Investig.* **8**, 31-37.

Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., *et al*. (2015) Estimating the Global Burden of Endemic Canine Rabies. *PLoS Negl. Trop. Dis.* **9**, e0003709.

Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D.T., Kaare, M., *et al.* (2009) Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biol.* **7**, e53.

Hanlon, C.A., and Childs, J.E. (2013) Epidemiology. *In* A.C. Jackson (ed.), *Rabies: Scientific Basis of the Disease and Its Management*. Academic Press.

Hanlon, C.A., Niezgoda, M., and Rupprecht, C.E. (2013) Rabies in terrestrial animals. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press, pp. 201-258.

Harrison, T.M., Mazet, J.K., Holekamp, K.E., Dubovi, E., Engh, A.L., *et al*. (2004) Antibodies to canine and feline viruses in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve. *J. Wildl. Dis.* 4**0**, 1-10.

Hayman, D.T., Bowen, R.A., Cryan, M., McCracken, G.F., O'Shea, T.J., *et al*. (2012c) Ecology of Zoonotic Infectious Diseases in Bats: Current Knowledge and Future Directions. *Zoon. Publ. Health*, 60, 2-21.

Hayman, D.T., Emmerich, P., Yu, M., Wang, L.F., Suu-Ire, R., *et al*. (2010) Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS One* **5**, e11978.
Hayman, D.T., Fooks, A.R., Horton, D., Suu-Ire, R., Breed, A.C., *et al*. (2008) Antibodies against Lagos bat virus in Megachiroptera from West Africa. *Emerg. Infect. Dis.* **14**, 926.
Hayman, D.T., Fooks, A.R., Marston, D.A., and Garcia-R, J.C. (2016) The Global Phylogeography of Lyssaviruses - Challenging the 'Out of Africa' Hypothesis. *PLoS Negl. Trop. Dis*. **10**, e0005266.
Hayman, D.T., Fooks, A.R., Rowcliffe, J.R., Restif, O., Baker, K.S., *et al*. (2012b) Endemic Lagos bat virus infection in *Eidolon helvum*. *Epidemiol. Infect.* **140**, 2163-2171.
Hayman, D.T., Johnson, N., Horton, D.L., Hedge, J., Wakeley, P.R., *et al*. (2011) Evolutionary history of rabies in Ghana. *PLoS Negl. Trop. Dis*. **5**, e1001.
Hayman, D.T., McCrea, R., Restif, O., Suu-Ire, R., Fooks, A.R., *et al*. (2012a) Demography of straw-colored fruit bats in Ghana. *J. Mammalol.* 93, 1393-1404.
Hedenström, A., and Johansson, L.C. (2015) Bat flight: aerodynamics, kinematics and flight morphology. *J. Exp. Biol.* **218**, 653-663.
Hemachudha, T., Laothamatas, J., and Rupprecht, C. E. (2002) Human rabies: A disease of complex neuropathogenetic mechanisms and diagnostic challenges. *Lancet Neurol*. **1**, 101-109.
Hemachudha, T., Ugolini, G., Wacharapluesadee, S., Sungkarat, W., Shuangshoti, S., *et al.* (2013) Human rabies: neuropathogenesis, diagnosis, and management. *Lancet Neurol.* **12**, 498-513.
Hemachudha, T., Wacharapluesadee, S., Mitrabhakdi, E., Wilde, H., Morimoto, K., *et al*. (2005) Pathophysiology of human paralytic rabies. *J. Neurovirol*. **11**, 93-100.
Herrera, G.L., Gutierrez, E., Hobson, K.A., Altube, B., Díaz, W.G., *et al.* (2002) Sources of assimilated protein in five species of New World frugivorous bats. *Oecologia* **133**, 280-287.
Ho, S.Y., Lanfear, R., Bromham, L., Phillips, M.J., Soubrier, J., *et al.* (2011) Time-dependent rates of molecular evolution. *Mol. Ecol*. **20**, 3087-3101.
Hofer, H., and East, M.L. (1995) Population dynamics, population size and the commuting system of Serengeti spotted hyenas. *In* A.R.E. Sinclair and P. Arcese (eds.), *Serengeti II. Dynamics, Management, and Conservation of an Ecosystem*. Chicago: Chicago Univ. Press, pp. 332-363.
Holmes, E.C. (2003) Error thresholds and the constraints to RNA virus evolution. *Trends Microbiol.* **11**, 543-546.
Holmes, E.C. (2008) Evolutionary History and Phylogeography of Human Viruses. *Annu. Revs. Microbiol.* **62**, 307-328.
Holmes, E.C. (2010) The RNA virus quasispecies: fact or fiction? *J. Mol. Bio.* **400**, 271-273.
Holmes, E.C. (2016) Complexities of estimating evolutionary rates in viruses. *J. Virol*. **90**, 2155.
Holmes, E.C., Woelk, C.H., Kassis, R., and Bourhy, H. (2002) Genetic constraints and the adaptive evolution of rabies virus in nature. *Virology* **292**, 247-257.
Horton, D.L., Banyard, A.C., Marston, D.A., Wise, E., Selden, D., *et al*. (2014) Antigenic and genetic characterization of a divergent African virus, Ikoma lyssavirus. *J. Gen. Virol*. **95**, 1025-1032.
Hu, R., Tang, Q., Tang, J., and Fooks, A.R. (2009) Rabies in China: an update. *Vect.-Borne Zoon. Dis.* 9, 1-12.
Hughes, G.J., Kuzmin, I.V., Schmitz, A., Blanton, J., Manangan, J., *et al*. (2006) Experimental infection of big brown bats (*Eptesicus fuscus*) with Eurasian bat lyssaviruses Aravan, Khujand, and Irkut virus. *Arch. Virol*. **151**, 2021-2035.

Hughes, G.J., Orciari, L.A., and Rupprecht, C.E. (2005) Evolutionary timescale of rabies virus adaptation to North American bats inferred from the substitution rate of the nucleoprotein gene. *J. Gen. Virol*. **86**, 1467-1474.

Huguin, M., Arechiga-Ceballos, N., Delaval, M., Guidez, A., Jorge de Castro, I., *et al*. (2017) How social structure drives the population dynamics of the common vampire bat (*Desmodus rotundus*, Phyllostomidae). *J. Hered.*

Jackson, A.C. (2007a) Human Disease. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press.

Jackson, A.C. (2007b) Pathogenesis. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press, pp. 341-382.

Jackson, A.C. (2008) Rabies. *Neurol. Clin*. **26**, 717-726.

Jackson, A.C. (2014) Rabies. *In* M. Scheld, R. J. Whitley, and C. Marra, (eds.), *Infections of the Central Nervous System*, 4th edn. pp. 243–259., Philadelphia: Lippincott, Williams and Wilkins.

Jackson, F.R., Turmelle, A.S., Farino, D.M., Franka, R., McCracken, G.F., *et al*. (2008) Experimental rabies virus infection of big brown bats (*Eptesicus fuscus*). *J. Wildl. Dis*. **44**, 612-621.

Johnson, N., Aréchiga-Ceballos, N., and Aguilar-Setien, A. (2014) Vampire Bat Rabies: Ecology, Epidemiology and Control. *Viruses* **6**, 1911-1928.

Johnson, N., Cunningham, A.F., and Fooks, A.R. (2010) The immune response to rabies virus infection and vaccination. *Vaccine* **28**, 3896-3901.

Johnson, N., Phillpotts, R., and Fooks, A.R. (2006b) Airborne transmission of lyssaviruses. *J. Med. Microbiol.* **55**, 785-790.

Johnson, N., Vos, A., Neubert, L., Freuling, C., Mansfield, K.L., *et al*. (2008) Experimental study of European bat lyssavirus type-2 infection in Daubenton's bats (*Myotis daubentonii*). *J. Gen. Virol.* **89**, 2662-2672.

Johnson, N., Wakeley, P.R., Brookes, S.M., and Fooks, A.R. (2006a) European bat lyssavirus type 2 RNA in *Myotis daubentonii*. *Emerg. Infect. Dis.* **12**, 1142.

Jones, G., and Siemers, B.M. (2011) The communicative potential of bat echolocation pulses. *J. Comp. Physiol. A* **197**, 447-457.

Juste, J., Benda, P., Garcia-Mudarra, J.L., and Ibáñez, C. (2013) Phylogeny and systematics of Old World serotine bats (genus *Eptesicus*, Vespertilionidae, Chiroptera): an integrative approach. *Zool. Scripta* **42**, 441-457.

Kading, R.C., Gilbert, A.T., Mossel, E.C., Crabtree, M.B., Kuzmin, I.V., *et al*. (2013) Isolation and molecular characterization of Fikirini rhabdovirus, a novel virus from a Kenyan bat. *J. Gen. Virol*. **94**, 2393-2398.

Kalemba, L.S.N., Niezgoda, M., Gilbert, A.T., Doty, J.B., Wallace, R.M., *et al*. (2017) Exposure to Lyssaviruses in Bats of the Democratic Republic of the Congo. *J. Wildl. Dis.* **53**, 408-410.

Kaplan, H.J., and Niederkorn, J.Y. (2007) Regional immunity and immune privilege. *Chem. Immunol. Allergy* **92**, 11-26.

Kgaladi J, Wright N, Coertse J, Markotter W, Marston D, *et al*. (2013) Diversity and Epidemiology of Mokola Virus. *PLoS Negl. Trop. Dis*. **7**, e2511.

Kia, G.S., Kuzmin, I., Umoh, J.U., Kwaga, J.K., Kazeem, H.M., *et al*. (2014) Detection of some lyssaviruses from fruigivorous and insectivorous bats in Nigeria. *Online J. Publ. Health Inform*. **6**, 1.

Kissi, B., Badrane, H., Audry, L., Lavenu, A., Tordo, N., *et al.* (1999) Dynamics of rabies virus quasispecies during serial passages in heterologous hosts. *J. Gen. Virol.* **80**, 2041-2050.

Knobel, D., Kaare, M., Fèvre, E., and Cleaveland, S. (2007) Dog Rabies and its Control. *In* A.C. Jackson and W.H. Wunner (eds.), *Rabies* (2nd edition). London: Academic Press.

Knobel, D.L., Fooks, A.R., Brookes, S.M., Randall, D.A., Williams, S.D., *et al*. (2007) Trapping and vaccination of endangered Ethiopian wolves to control an outbreak of rabies. *J. Appl. Ecol.* **45**, 109-116.

Knörnschild, M., Jung, K., Nagy, M., Metz, M., and Kalko, E. (2012) Bat echolocation calls facilitate social communication. *Proc. Roy. Soc. Lond. Ser. B* **279**, 4827-4835.

Kobayashi, M. (2017) Homology of the muscles within the uropatagium membrane in Leschenault's rousette (*Rousettus leschenaultii*). *Mamm. Biol.* **86**, 102-106.

Kobayashi, Y., Sato, G., Mochizuki, N., Hirano, S., Itou, T., *et al*. (2008) Molecular and geographic analyses of vampire bat-transmitted cattle rabies in central Brazil. *BMC Vet. Res*. **4**, 44.

Koyuncu, O.O., Hogue, I.B., and Enquist, L.W. (2013) Virus infections in the nervous system. *Cell Host Microbe* **13**, 379-393.

Kunz, T.H. (1982) Roosting ecology of bats. *In* T.H. Kunz (ed.), *Ecology of Bats*. Plenum, pp. 1-55.

Kuzmin, I.V., and Rupprecht, C.E. (2007) Bat Rabies. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press.

Kuzmin, I.V., Bozick, B., Guagliardo, S.A., Kunkel, R., Shak, J.R., *et al.* (2011) Bats, emerging infectious diseases, and the rabies paradigm revisited. *Emerg. Health Threats J*. **4**, 7159.

Kuzmin, I.V., Franka, R., and Rupprecht, C.E. (2008e) Experimental infection of big brown bats (*Eptesicus fuscus*) with West Caucasian bat virus (WCBV). *Dev. Biol. (Basel)* **131**, 327-337 [abstract].

Kuzmin, I.V., Hughes, G.J., Botvinkin, A.D., Gribencha, S.G., and Rupprecht, C.E. (2008b) Arctic and arctic-like rabies viruses: distribution, phylogeny and evolutionary history. *Epidemiol. Infect*. **136**, 509-519.

Kuzmin, I.V., Mayer, A.E., Niezgoda, M., Markotter, W., Agwanda, B., *et al*. (2010) Shimoni bat virus, a new representative of the Lyssavirus genus. *Virus Res*. **149**, 197-210.

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., *et al*. (2008d) Possible emergence of West Caucasian bat virus in Africa. *Emerg. Infect. Dis.* **14**, 1887.

Kuzmin, I.V., Niezgoda, M., Franka, R., Agwanda, B., Markotter, W., *et al*. (2008c) Lagos bat virus in Kenya. *J. Clin. Microbiol.* **46**, 1451-1461.

Kuzmin, I.V., Novella, I.S., Dietzgen, R.G., Padhi, A., and Rupprecht, C.E. (2009) The rhabdoviruses: biodiversity, phylogenetics, and evolution. *Infect. Genet. Evol.* 9, 541-553.

Kuzmin, I.V., Shi, M., Orciari, L.A., Yager, P.A., Velasco-Villa, A., *et al.* (2012) Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. *PLoS Pathog*. **8**, e1002786.

Kuzmin, I.V., Turmelle, A.S., Agwanda, B., Markotter, W., Niezgoda, M., *et al*. (2011) Commerson's leaf-nosed bat (*Hipposideros commersoni*) is the likely reservoir of Shimoni bat virus. *Vect.-Borne Zoon. Dis.* **11**, 1465-1470.

Kuzmin, I.V., Wu, X., Tordo, N., and Rupprecht, C.E. (2008a) Complete genomes of Aravan, Khujand, Irkut and West Caucasian bat viruses, with special attention to the polymerase gene and non-coding regions. *Virus Res*. **136**, 81-90.

Kuzmina, N.A., Kuzmin, I.V., Ellison, J.A., Taylor, S.T., Bergman, D.L., *et al.* (2013) A reassessment of the evolutionary timescale of bat rabies viruses based upon glycoprotein gene sequences. *Virus Genes* **47**, 305-310.

Kyle, C.J., Rico, Y., Castillo, S., Srithayakumar, V., Cullingham, C.I., White, B.N. *et al*. (2014) Spatial patterns of neutral and functional genetic variations reveal patterns of local adaptation in raccoon (*Procyon lotor*) populations exposed to raccoon rabies. *Mol. Ecol*. **23**, 2287-2298.

Lackay, S.N., Kuang, Y., and Fu, Z.F. (2008) Rabies in small animals. *Vet. Clin. North Amer. Small Anim. Pract.* **38**, 851-861.
Lafon, M. (2005) Rabies virus receptors. *J. Neurovirol*. **11**, 82-87.
Lafon, M. (2008) Immune evasion, a critical strategy for rabies virus. *Dev. Biol. (Basel)* **131**, 413-419.
Lafon, M. (2011) Evasive strategies in rabies virus infection. *Adv. Virus Res*. **79**, 33-53.
Lafon, M. (2016) Rabies. *In* C.S. Reiss (ed.), *Neurotropic Viral Infections*. *Volume 1: Neurotropic RNA Viruses*. Springer, pp. 85-113.
Lan, Y.C., Wen, T.H., Chang, C.C., Liu, H.F., Lee, P.F., *et al.* (2017) Indigenous Wildlife Rabies in Taiwan: Ferret Badgers, a Long Term Terrestrial Reservoir. *BioMed Res. Internat.*
Laothamatas, J., Wacharapluesadee, S., Lumlertdacha, B., Ampawong, S., Tepsumethanon, V., *et al*. (2008) Furious and paralytic rabies of canine origin: neuroimaging with virological and cytokine studies. *J. Neurovirol*. **14**, 119-129.
Lembo, T., Haydon, D.T., Velasco-Villa, A., Rupprecht, C.E., Packer, C., *et al.* (2007) Molecular epidemiology identifies only a single rabies virus variant circulating in complex carnivore communities of the Serengeti. *Proc. Roy. Soc. Lond. Ser. B* **274**, 2123-2130.
Lembo, T., Hampson, K., Haydon, D.T., Craft, M., Dobson, A., *et al.* (2008) Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. *J. Appl. Ecol.* **45**, 1246-1257.
Leonard, J.A., Wayne, R.K., Wheeler, J., Valadez, R., Guillén, S., *et al.* (2002) Ancient DNA evidence for Old World origin of New World dogs. *Science* **298**, 1613-1616.
Leslie, M. J., Messenger, S., Rohde, R. E., Smith, J., Cheshier, R., *et al.* (2006) Bat-associated Rabies Virus in Skunks. *Emerg. Infect. Dis.* **12**, 1274-1277.
Lewanzik, D., Kelm, D.H., Greiner, S., Dehnhard, M., and Voigt, C.C. (2012) Ecological correlates of cortisol levels in two bat species with contrasting feeding habits. *Gen. Comp. Endocr*. **177**, 104-112.
Li, C.X., Shi, M., Tian, J.H., Lin, P.D., Kang, Y.J., *et al.* (2015a) Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Elife* **4**, e05378.
Li, C.X., Shi, M., Tian, J.H., Lin, P.D., Kang, Y.J., *et al.* (2015b) Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Supplementary information. *Elife* **4**, e05378.
Liu, Y., Zhang, S., Wu, X., Zhao, J., Hou, Y., *et al.* (2010) Ferret badger rabies origin and its revisited importance as potential source of rabies transmission in Southeast China. BMC Infect. Dis. 10, 234.
Lodmell, D.L., Dimcheff, D.E., and Ewalt, L.C. (2006) Viral RNA in the bloodstream suggests viremia occurs in clinically ill rabies-infected mice. *Virus Res.* **116**, 114-118.
Longdon, B., Murray, G.G., Palmer, W.J., Day, J.P., Parker, D.J., *et al.* (2015) The evolution, diversity, and host associations of rhabdoviruses. *Virus Evol.* **1**, 1.
Lopez, R.A., Miranda, P.P., Tejada, V.E., and Fishbein, D.B. (1992) Outbreak of human rabies in the Peruvian jungle. *Lancet* **339**, 408-411.
López-Roig, M., Bourhy, H., Lavenir, R., and Serra-Cobo, J. (2014) Seroprevalence dynamics of European bat lyssavirus type 1 in a multispecies bat colony. *Viruses* **6**, 3386-3399.
Lord, R.D. (1980) *An ecological strategy for controlling bovine rabies through elimination of vampire bats.* Proceedings of the 9th Vertebrate Pest Conference, Paper 22.
Lord, R.D. (1992) Seasonal Reproduction of Vampire Bats and Its Relation to Seasonality of Bovine Rabies. *J. Wildl. Dis.* 28, 292-294.

Lu, Z.L., Wang, W., Yin, W.L., Tang, H.B., Pan, Y., *et al.* (2013) Lyssavirus surveillance in bats of Southern China's Guangxi province. *Virus Genes* **46**, 293-301.
Luo, B., Huang, X., Li, Y., Lu, G., Zhao, J., *et al.* (2017) Social call divergence in bats: a comparative analysis. *Behav. Ecol.* **28**, 533-540.
Lyman, C.P. (1982) Thermoregulation and Metabolism in Bats. *In* T.H. Kunz (ed.), *Ecology of Bats*. Plenum, pp. 301-359.
MacHugh, D.E., Larson, G., and Orlando, L. (2017) Taming the past: ancient DNA and the study of animal domestication. *Ann. Rev. Anim. Biosci.* **5**, 329-351.
Mackenzie, J.S., Childs, J.E., Field, H.E., Wang, L.-F., and Breed, A.C. (2016) The Role of Bats as Reservoir Hosts of Emerging Neuroviruses. *In* C.S. Reiss (ed.), *Neurotropic Viral Infections*. *Volume 2: Neurotropic Retroviruses, DNA Viruses, Immunity and Transmission*. Springer, pp. 403-454.
Madej, J.P., Mikulová, L., Gorošová, A., Mikula, Š., Řehák, Z., *et al*. (2013) Skin structure and hair morphology of different body parts in the Common Pipistrelle (*Pipistrellus pipistrellus*). *Acta Zool.* **94**, 478-489.
Makanya, A.N., and Mortola, J.P. (2007) The structural design of the bat wing web and its possible role in gas exchange. *J. Anat*. **211**, 687-697.
Manning, S.E., Rupprecht, C.E., Fishbein, D., Hanlon, C.A., Lumlertdacha, B., *et al*. (2008) Human rabies prevention — United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *Morb. Mortal. Wkly. Rept.* **57**, 1-28.
Markotter, W., Kuzmin, I., Rupprecht, C.E., and Nel, L.H. (2008) Phylogeny of Lagos bat virus: Challenges for lyssavirus taxonomy. *Virus Res*. **135**, 10-21.
Markotter, W., Kuzmin, I., Rupprecht, C.E., Randles, J., Sabeta, C.T., *et al*. (2006) Isolation of Lagos bat virus from water mongoose. *Emerg. Infect. Dis.* **12**, 1913-1917.
Markotter, W., Kuzmin, I.V., Rupprecht, C.E., and Nel, L.H. (2009) Lagos bat virus virulence in mice inoculated by the peripheral route. *Epidemiol. Infect*. **137**, 1155-1162.
Markotter, W., Monadjem, A., and Nel, L.H. (2013) Antibodies against Duvenhage Virus in insectivorous bats in Swaziland. *J. Wildl. Dis.* **49**, 1000-1003.
Marshall, A.G. (1982) Ecology of Insects Ectoparasitic on Bats. *In* T.H. Kunz (ed.), *Ecology of Bats*. New York: Plenum, pp. 369-402.
Marshall, K.L., Chadha, M., Sterbing-D'Angelo, S.J., Moss, C.F., and Lumpkin, E.A. (2015) Somatosensory substrates of flight control in bats. *Cell Repts.* **11**, 851-858.
Marshall-Pescini, S., Schwarz, J.F., Kostelnik, I., Virányi, Z., and Range, F. (2017) Importance of a species' socioecology: Wolves outperform dogs in a conspecific cooperation task. *Proc. Nat. Acad. Sci. USA* **114**, 11793-11798.
Marston, D.A., Banyard, A.C., McElhinney, L.M., Freuling, C.M., Finke, S., *et al.* (2018) The lyssavirus host-specificity conundrum – Rabies virus – The exception not the rule. *Curr. Opin. Virol.* **28**, 68-73.
McColl, K., Chamberlain, T., Lunt, R., Newberry, K., Middleton, D., *et al.* (2002) Pathogenesis studies with Australian bat lyssavirus in grey-headed flying foxes (*Pteropus poliocephalus*). *Aust. Vet. J.* **80**, 636–641.
McColl, K.A., Chamberlain, T., Lunt, R.A., Newberry, K.M., and Westbury, H.A. (2007) Susceptibility of domestic dogs and cats to Australian bat lyssavirus (ABLV). *Vet. Microbiol.* **123**, 15-25.
McColl, K.A., Tordo, N., and Aguilar, S.A. (2000) Bat lyssavirus infections. *Rev. Sci. Tech.* **19**, 177-196.
McCollum, A.M., Blanton, J.D., Holman, R.C., Callinan, L.S., Baty, S., *et al.* (2012) Community survey after rabies outbreaks, Flagstaff, Arizona, USA. *Emerg. Infect. Dis.* **18**, 932-938.

McElhinney, L.M., Marston, D.A., Leech, S., Freuling, C.M., van der Poel, W.H.M., *et al*. (2013) Molecular epidemiology of bat lyssaviruses in Europe. *Zoon. Publ. Health* **60**, 35-45.

Meng, S., Sun, Y., Wu, P., Tang, J., Xu, G., *et al*. (2011) Evolutionary dynamics of rabies viruses highlights the importance of China rabies transmission in Asia. *Virol.* **410**, 403-409.

Messenger, S.L., Smith, J.S., and Rupprecht, C.E. (2003) Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. *Clin. Infect. Dis.* **35**, 738-747.

Miller-Butterworth, C.M., Jacobs, D.S., and Harley, E.H. (2003) Strong population substructure is correlated with morphology and ecology in a migratory bat. *Nature* **424**, 187-191.

Minard, A. (2009) New, Fast-Evolving Rabies Virus Found - And Spreading. *Nat. Geog. News* http://news.nationalgeographic.com/news/2009/05/090504-rabies-evolution.html.

Mollentze, N., Biek, R., and Streicker, D.G. (2014) The role of viral evolution in rabies host shifts and emergence. *Curr. Opin. Virol.* **8**, 68-72.

Moore, S.M., and Hanlon, C.A. (2010) Rabies-specific antibodies: measuring surrogates of protection against a fatal disease. *PLoS Negl. Trop. Dis.* **4**, e595.

Moore, S.M., and Hanlon, C.A. (2010) Rabies-specific antibodies: Measuring surrogates of protection against a fatal disease. *PLoS Negl. Trop. Dis.* **49**, 1-6.

Moore, S.M., Gilbert, A., Vos, A., Freuling, C.M., Ellis, C., *et al.* (2017) Rabies virus antibodies from oral vaccination as a correlate of protection against lethal infection in wildlife. *Trop. Med. Infect. Dis.* **2**, 31.

Mori, I., Nishiyama, Y., Yokochi, T., and Kimura, Y. (2005) Olfactory transmission of neurotropic viruses. *J. Neurovirol.* **11**, 129-137.

Morimoto, K., Hooper, D.C., Carbaugh, H., Fu, Z.F., Koprowski, H., *et al*. (1998) Rabies virus quasispecies: implications for pathogenesis. *Proc. Nat. Acad. Sci. USA* **95**, 3152-3156.

Morimoto, K., Patel, M., Corisdeo, S., Hooper, D.C., Fu, Z.F., *et al.* (1996) Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in North America. *Proc. Nat. Acad. Sci. USA* **93**, 5653-5658.

Mørk, T., and Prestrud, P. (2004) Arctic rabies – a review. *Acta Vet. Scand.* **45**, 1.

Mqokeli, B.R., and Downs, C.T. (2014) Is protein content in the diet of Wahlberg's epauletted fruit bats, *Epomophorus wahlbergi*, important? *Afr. Zool.* **49**, 161-166.

Mshelbwala, P.P., Ogunkoya, A.B., and Maikai, B.V. (2013) Detection of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption and its public health implications in Abia State, Nigeria. *ISRN Vet. Sci.* **2013**.

Mungrue, K., and Mahabir, R. (2011) The Rabies epidemic in Trinidad of 1923 to 1937: An evaluation with a geographic information system. *Wild. Env. Med.* **22**, 28-36.

Munro, A.D. (2017) A brief overview of the history of human communicable diseases and potential future zoonotic threats with particular reference to viruses and tuberculosis. *Univ. Cambod. Monogr. Ser*. **1**, 1-315.

Murphy, F.A., Bell, J.F., Bauer, S.P., Gardner, J.J., Moore, G.J., *et al*. (1980) Experimental chronic rabies in the cat. *Lab. Invest*. **43**, 231-241. [Abstract]

Nadin-Davis, S.A., and Real, L.A. (2011) Molecular phylogenetics of the lyssaviruses – insights from a coalescent approach. *Adv. Virus Res*. **79**, 203-238.

Nadin-Davis, S.A., Sheen, M., and Wandeler, A.I. (2012) Recent emergence of the Arctic rabies virus lineage. *Virus Res.* **163**, 352-362.

Nadin-Davis, S.A., Velez, J., Malaga, C., and Wandeler, A.I. (2008) A molecular epidemiological study of rabies in Puerto Rico. *Virus Res*. 131, 8-15.

Nadine-Davis, S.A., and Bingham, J. (2004) Europe as a source of rabies for the rest of the world. *In* A.A. King, A.R. Fooks, M. Aubert and A.I. Wandeler (eds.), *Historical perspective of Rabies in Europe and the Mediterranean Basin*. Paris, France: OIE. pp. 259-280.

Naish, D. (2007a) Desmodontines: the amazing vampire bats. http://www.scienceblogs.com/tetrapodzoology/2007/02/08/desmodontines-the-amazing-vamp/.

Naish, D. (2007b) Dark origins: the mysterious evolution of blood-feeding in bats. http://www.scienceblogs.com/tetrapodzoology/2007/02/16/dark-origins-the-mysterious-ev/.

Naish, D. (2007c) A new hypothesis on the evolution of blood-feeding: food source duality involving nectarivory. Catchy, no? http://scienceblogs.com/tetrapodzoology/2007/02/17/a-new-hypothesis-on-the-evolut/.

Naish, D. (2011a) Putting the 'perimyotines' well away from pips proper (vesper bats part XII). http://scienceblogs.com/tetrapodzoology/2011/04/12/placing-perimyotines/

Naish, D. (2011b) Introducing the *second* largest mammalian 'family': vesper bats, or vespertilionids. http://www.scienceblogs.com/tetrapodzoology/2011/03/16/introducing-vesper-bats/

Naish, D. (2011c) The vesper bat family tree: of myotines, plecotins, antrozoins, and all those cryptic species (vesper bats part II). http://www.scienceblogs.com/tetrapodzoology/2011/03/17/vesper-bat-family-tree/

Naish, D. (2011d) Bent-winged bats: wide ranges, very weird wings (vesper bats part III). http://www.scienceblogs.com/tetrapodzoology/2011/03/19/bent-winged-bats/

Naish, D. (2013) What did giant extinct vampire bats eat? https://blogs.scientificamerican.com/tetrapod-zoology/what-did-giant-extinct-vampire-bats-eat/.

Nel, L.H., and Rupprecht, C.E. (2007) Emergence of lyssaviruses in the Old World: the case of Africa. *In* J.E. Childs, J.S. Mackenzie and J.A. Richt (eds.), *Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission*. Berlin: Springer, pp. 161-193.

Norberg, U.M. (1972) Functional osteology and myology of the wing of the dog-faced bat *Rousettus aegyptiacus* (E. Geoffroy) (Mammalia, Chiroptera). *Z. Morph. Tiere* **73**, 1-44.

O'Mara, M.T., Rikker, S., Wikelski, M., ter Maat, A., Pollock, H.S., *et al*. (2017b) Heart rate reveals torpor at high body temperatures in lowland tropical free-tailed bats. *Roy. Soc. Open Sci.* **4**, 171359.

O'Mara, M.T., Wikelski, M., Voigt, C.C., ter Maat, A., Pollock, H.S., *et al.* (2017a) Cyclic bouts of extreme bradycardia counteract the high metabolism of frugivorous bats. *eLife* **6**, e26686.

O'Shea, T.J., Bowen, R.A., Stanley, T.R., Shankar, V., and Rupprecht, C.E. (2014a) Variability in seroprevalence of rabies virus neutralizing antibodies and associated factors in a Colorado population of big brown bats (*Eptesicus fuscus*). *PLoS One* **9**, e86261.

O'Shea, T.J., Cryan, P.M., Cunningham, A.A., Fooks, A.R., Hayman, D.T., *et al.* (2014b) Bat flight and zoonotic viruses. *Emerg. Inf. Dis.* **20**, 741.

O'Shea, T.J., Cryan, M., Hayman, D.T.S., Plowright, R.K., and Streicker, D.G. (2016a) Multiple mortality events in bats: A global review. *Mammal Revs*. **46**, 175-190.

O'Shea, T.J., Cryan, M., Hayman, D.T.S., Plowright, R.K., and Streicker, D.G. (2016b) Multiple mortality events in bats: A global review - Appendix S7, Reports of multiple bat deaths due to or suggestive of infectious viral or bacterial disease. *Mammal Revs*. **46**, 175-190.

Obregón-Morales, C., Aguilar-Setién, Á., Martínez, L.P., Galvez-Romero, G., Martínez-Martínez, F.O., *et al*. (2017) Experimental infection of *Artibeus intermedius* with a vampire bat rabies virus. *Comp. Immunol. Microbiol. Inf. Dis.* **52**, 43-47.

Olival, K.J., Epstein, J.H., Wang, L.F., Field, H.E., and Daszak, P. (2012) Are bats exceptional viral reservoirs? *In* A.A. Aguirre, R.S. Ostfeld and P. Daszak, (eds.), *New Directions in Conservation Medicine: Applied Cases of Ecological Health*. Oxford: Oxford University Press, pp. 195-212.

Olival, K.J., Weekley, C.C., and Daszak, P. (2015) Are bats really "special" as viral reservoirs? What we know and need to know. *In* L.-F. Wang and C. Cowled (eds.), *Bats and Viruses: A New Frontier of Emerging Infectious Diseases*. John Wiley and Sons, Inc. Oxford University Press, pp. 195-21.

Oliveira, R.N., de Souza, S.P., Lobo, R.S.V., Castilho, J.G., Macedo, C.I., *et al*. (2010) Rabies virus in insectivorous bats: implications of the diversity of the nucleoprotein and glycoprotein genes for molecular epidemiology. *Virology* **405**, 352-360.

Otolorin, G.R., Umoh, J.U., and Dzikwi, A.A. (2014) Prevalence of rabies antigen in brain tissue of dogs slaughtered for human consumption and evaluation of vaccination of dogs against rabies in Aba, Abia state Nigeria. *Sciences, 3*(1), pp.5-10.

Pajamo, K., Harkess, G., Goddard, T., Marston, D., McElhinney, L., *et al*. (2008) Isolation of European bat lyssavirus type 2 (EBLV-2) in a Daubenton's bat in the UK with a minimum incubation period of 9 months. *Rabies Bull. Europe* **32** (2), 6-7.

Parker, D.I., Lawhead, B.E., and Cook, J.A. (1997) Distributional limits of bats in Alaska. *Arctic* **50,** 256-265.

Parrish, N.F., and Tomonaga, K. (2018) A Viral (Arc)hive for Metazoan Memory. *Cell* **172**, 8-11.

Peel, A.J., Sargan, D.R., Baker, K.S., Hayman, D.T.S., Barr, J.A., *et al*. (2013) Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. *Nature Comms.* **4**, 2770.

Pérez-Jordá, J.L., Ibáñez, C., Muñoz-Cervera, M., and Téllez, A. (1995) Lyssavirus in *Eptesicus serotinus* (Chiroptera: Vespertilionidae). *J. Wildl. Dis.* **31**, 372-377.

Perl, D.P., Bell, J.F. and Moore, G.J. (1977). Chronic recrudescent rabies in a cat. *Proc. Soc. Exp. Biol. Med*. **155**, 540-548. [Abstract]

Pfalzer, G., and Kusch, J. (2003) Structure and variability of bat social calls: implications for specificity and individual recognition. *J. Zool*. **261**, 21-33.

Picard-Meyer, E., Servat, A., Robardet, E., Moinet, M., Borel, C., *et al*. (2013) Isolation of Bokeloh bat lyssavirus in *Myotis nattereri* in France. *Arch. Virol*. **158**, 2333-2340.

Platt, R.N., Faircloth, B.C., Sullivan, K.A., Kieran, T.J., Glenn, T.C., *et al.* (2017) Conflicting Evolutionary Histories of the Mitochondrial and Nuclear Genomes in New World *Myotis* Bats. *Systemat. Biol*.

Pollock, T., Moreno, C.R., Sánchez, L., Ceballos-Vasquez, A., Faure, P.A., *et al.* (2016) Wound healing in the flight membranes of wild big brown bats. *J. Wildl. Man.* **80**, 9-26.

Pons-Salort, M., Serra-Cobo, J., Jay, F., Lopez-Roig, M., Lavenir, R., *et al*. (2014) Insights into persistence mechanisms of a zoonotic virus in bat colonies using a multispecies metapopulation model. *PLoS One* **9**, e95610.

Prager, K.C., Mazet, J.A., Dubovi, E.J., Frank, L.G., Munson, L., *et al.* (2012) Rabies virus and canine distemper virus in wild and domestic carnivores in Northern Kenya: are domestic dogs the reservoir? *EcoHealth, 9*(4), pp.483-498.

Preuss, M.A., Faber, M.L., Tan, G.S., Bette, M., Dietzschold, B., *et al*. (2009) Intravenous inoculation of a bat-associated rabies virus causes lethal encephalopathy in mice through invasion of the brain *via* neurosecretory hypothalamic fibers. *PLoS Pathog*. **5**, e1000485.

Pybus, O.G., and Thézé, J. (2016) Hepacivirus cross-species transmission and the origins of the hepatitis C virus. *Curr. Opin. Virol.* **16**, 1-7.
Pybus, O.G., Markov, P.V., Wu, A., and Tatem, A.J. (2007) Investigating the endemic transmission of the hepatitis C virus. *Int. J. Parasitol*. **37**, 839-849.
Quammen, D. (2014) Excerpt from *Spillover: Animal Infections and the Next Human Pandemic. The Geographical Pattern of Ebola Outbreaks*. https://medium.com/science-and-technology/the-geographical-pattern-of-ebola-outbreaks-9bc796a4cbfe#.xlva61icm.
Ramsden, R.O., and Johnson, D.H. (1975) Studies on the oral infectivity of rabies virus in Carnivora. *J. Wildl. Dis*. **11**, 318-324.
Randall, D.A., Williams, S.D., Kuzmin, I.V., Rupprecht, C.E., Tallents, L.A., *et al*. (2004) Rabies in endangered Ethiopian wolves. *Emerg. Infect. Dis*. **10**, 2214-2217.
Raundrup, K., Moshøj, C.M., Wennerberg, S.E., and Kapel, C.M. (2015) Spatiotemporal distribution of rabies in Arctic foxes in Greenland. *Eur. J. Wildl. Res.* **61**, 457-465.
Reeder, D.M., Kosteczko, N.S., Kunz, T.H., and Widmaier, E.P. (2004) Changes in baseline and stress-induced glucocorticoid levels during the active period in free-ranging male and female little brown myotis, *Myotis lucifugus* (Chiroptera: Vespertilionidae). *Gen. Comp. Endocr*. **136**, 260-269.
Reid, J.E., and Jackson, A.C. (2001) Experimental rabies virus infection in *Artibeus jamaicensis* bats with CVS-24 variants. *J. Neurovirol*. 7, 11-17.
Reynes, J.-M., Molia, S., Audry, L., Hout, S., Ngin, S., *et al*. (2004) Serologic evidence of Lyssavirus infection in bats, Cambodia. *Emerg. Infect. Dis.* **10**, 2231-2234.
Rodríguez-Nevado, C., Lam, T.T.Y., Holmes, E.C., and Pagán, I. (2018) The impact of host genetic diversity on virus evolution and emergence. *Ecol. Letters* **21**, 253-263.
Rønn, L.C.B., Hartz, B.P., and Bock, E. (1998) The neural cell adhesion molecule (NCAM) in development and plasticity of the nervous system. *Exp. Gerontol.* **33**, 853-864.
Ronsholt, L., Sorensen, K.J., Bruschke, C.J.M., Wellenberg, G.J., van Oirschot, J.T., *et al*. (1998) Clinical silent rabies infection in (zoo) bats. *Vet. Rec*. **142**, 519-20.
Rosseels, V., Nazé, F., De Craeye, S., Francart, A., Kalai, M., *et al*. (2011) A non-invasive intranasal inoculation technique using isoflurane anesthesia to infect the brain of mice with rabies virus. *J. Virol. Meth*. **173**, 127-136.
Ruedi, M., and Mayer, F. (2001) Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Mol. Phylogenet. Evol.* **21**, 436-448.
Ruedi, M., Stadelmann, B., Gager, Y., Douzery, E.J., Francis, C.M., *et al.* (2013) Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). *Mol. Phylog. Evol.* **69**, 437-449.
Rupprecht, C., Kuzmin, I., and Meslin, F. (2017) Lyssaviruses and rabies: current conundrums, concerns, contradictions and controversies. *F1000Res* **6**, 184.
Rupprecht, C.E., Turmelle, A., and Kuzmin, I.V. (2011) A perspective on lyssavirus emergence and perpetuation. *Curr. Opin. Virol*. **1**, 662-670.
Russell, A.L., Pinzari, C.A., Vonhof, M.J., Olival, K.J., and Bonaccorso, F.J. (2015) Two Tickets to Paradise: Multiple Dispersal Events in the Founding of Hoary Bat Populations in Hawai'i. *PLoS One* **10**, e0127912.
Sadler, W.W., and Enright, J.B. (1959) Effect of metabolic level of the host upon the pathogenesis of Rabies in the bat. *J. Infect. Dis.* **105**, 267-273.
Schatz, J., Fooks, A.R., McElhinney, L., Horton, D., Echevarria, J., *et al*. (2012) Bat Rabies surveillance in Europe. *Zoon. Publ. Health*. 60, 22–34.
Schmidt-Chanasit, J., and Sauerbrei, A. (2011) Evolution and world-wide distribution of varicella-zoster virus clades. *Infect. Genet. Evol.* **11**, 1-10.

Schnell, M.J., McGettigan, J.P., Wirblich, C., and Papaneri, A. (2010) The cell biology of rabies virus: using stealth to reach the brain. *Nature Revs. Microbiol.* **8**, 51-61.

Schountz, T., Baker, M.L., Butler, J., and Munster, V. (2017) Immunological Control of Viral Infections in Bats and the Emergence of Viruses Highly Pathogenic to Humans. *Front. Immunol*. **8**.

Scott, T.P., and Nel, L.H. (2016) Subversion of the immune response by rabies virus. *Viruses* **8**, 231.

Seetahal, J.F., Vokaty, A., Carrington, C.V., Adesiyun, A.A., Mahabir, R., *et al*. (2017) The history of rabies in Trinidad: Epidemiology and control measures. *Trop. Med. Infect. Dis.* **2**, 27.

Serra-Cobo, J., Amengual, B., Abellán, C., and Bourhy, H. (2002) European bat lyssavirus infection in Spanish bat populations. *Emerg. Infect. Dis*. **8**, 413-420.

Serra-Cobo, J., López-Roig, M., Seguí, M., Sánchez, L.P., Nadal, J., *et al*. (2013) Ecological factors associated with European bat Lyssavirus seroprevalence in Spanish bats. *PLoS One* **8**, e64467.

Shankar, V., Bowen, R.A., Davis, A.D., Rupprecht, C.E., and O'Shea, T.J. (2004) Rabies in a colony of big brown bats (*Eptesicus fuscus*). *J. Wildl. Dis.* **40**, 403-413.

Shih, T.H., Wallace, R., and Wu, H.Y. (2017) The Progression of Taiwan Ferret Badger Rabies from July 2013 to December 2016. *J. Zoonot. Dis. Publ. Health* **1**, 3.

Shope, R.E. (1982) Rabies-Related Viruses. *Yale J. Biol. Med*. **55**, 271-275

Shuangshoti, S., Thorner, P.S., Teerapakpinyo, C., Thepa, N., Phukpattaranont, P., *et al*. (2016) Intracellular spread of rabies virus is reduced in the paralytic form of canine rabies compared to the furious form. *PLoS Negl. Trop. Dis.* **10**, e0004748.

Sitprija, V., Sriaroon, C., Lumlertdaecha, B., Wacharapluesadee, S., Phumesin, P., *et al*. (2003) Does contact with urine and blood from a rabid dog represent a rabies risk? *Clin. Infect. Dis*. **37**, 1399-1400.

Smith, J.S., Fishbein, D.B., Rupprecht, C.E., and Clark, K. (1991) Unexplained rabies in three immigrants in the United States. A virologic investigation. *NEJM* **324**, 205-211.

Smith, J.S., Orciari, L.A., Yager, A., Seidel, H.D., and Warner, C.K. (1992) Epidemiologic and historical relationships among 87 Rabies virus isolates as determined by limited sequence analysis. *J. Infect. Dis.* **166**, 296-307.

Srithayakumar, V., Castillo, S., Rosatte, R.C., and Kyle, C.J. (2011) MHC class II DRB diversity in raccoons (*Procyon lotor*) reveals associations with raccoon rabies virus (*Lyssavirus*). *Immunogenetics* **63**, 103-113.

Srithayakumar, V., Sribalachandran, H., Rosatte, R., Nadin-Davis, S.A., and Kyle, C.J. (2014) Innate immune responses in raccoons after raccoon rabies virus infection. *J. Gen. Virol*. **95**, 16-25.

Stadelmann, B., Lin, L.K., Kunz, T.H., and Ruedi, M. (2007) Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Mol. Phylog. Evol.* **43**, 32-48.

Steece, R., and Altenbach, J.S. (1989) Prevalence of rabies specific antibodies in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*) at Lava Cave, New Mexico. *J. Wildl. Dis*. **25**, 490-496.

Steece, R.S., and Calisher, C.H. (1989) Evidence for prenatal transfer of rabies virus in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). *J. Wildl. Dis.* **25**, 329-334.

Sterbing-d'Angelo, S., Chadha, M., Chiu, C., Falk, B., Xian, W., *et al*. (2011) Bat wing sensors support flight control. *Proc. Nat. Acad. Sci. USA* **108**, 11291-11296.

Streicker, D.G., Altizer, S.M., Velasco-Villa, A., and Rupprecht, C.E. (2012a) Variable evolutionary routes to host establishment across repeated rabies virus host shifts among bats. *Proc. Nat. Acad. Sci. USA* **109**, 19715-19720.

Streicker, D.G., Lemey, Velasco-Villa, A., and Rupprecht, C.E. (2012b) Rates of viral evolution are linked to host geography in bat rabies. *PLoS Pathog.* 8, e1002720.

Streicker, D.G., Recuenco, S., Valderrama, W., Gomez Benavides, J., Vargas, I., *et al*. (2012c) Ecological and anthropogenic drivers of rabies exposure in vampire bats: Implications for transmission and control. *Proc. Roy. Soc. Lond. Ser. B Biol. Sci.* 279, 3384–3392.

Streicker, D.G., Turmelle, A.S., Vonhof, M.J., Kuzmin, I.V., McCracken, G.F., *et al*. (2010) Host phylogeny constrains cross-species emergence and establishment of Rabies virus in bats. *Science* 329, 676-679.

Streicker, D.G., Winternitz, J.C., Satterfield, D.A., Condori-Condori, R.E., Broos, A., *et al*. (2016) Host-pathogen evolutionary signatures reveal dynamics and future invasions of vampire bat rabies. *Proc. Nat. Acad. Sci. USA* **113**, 10926-10931.

Suu-Ire, R.D., Fooks, A.R., Banyard, A.C., Selden, D., Amponsah-Mensah, K., *et al*. (2017) Lagos bat virus infection dynamics in free-ranging straw-colored fruit bats (*Eidolon helvum*). *Trop. Med. Infect. Dis.* **2**, 25.

Swanson, P.A., and McGavern, D.B. (2015) Viral diseases of the central nervous system. *Curr. Opin. Virol*. **11C**, 44-54.

Swartz, S.M., and Konow, N. (2015) Advances in the study of bat flight: the wing and the wind. *Can. J. Zool.* **93**, 977-990.

Swartz, S.M., Groves, M.S., Kim, H.D. and Wals, W.R. (1996) Mechanical properties of bat wing membrane skin. *J. Zool. (Lond.)* **239**, 357-378.

Swartz, S.M., Iriarte-Díaz, J., Riskin, D.K., and Breuer, K.S. (2012) A bird? A plane? No, it's a bat: an introduction to the biomechanics of bat flight. In *Evolutionary history of bats: fossils, molecules and morphology*. Cambridge University Press.

Takayama-Ito, M., Inoue, K.I., Shoji, Y., Inoue, S., Iijima, T., *et al*. (2006b) A highly attenuated rabies virus HEP-Flury strain reverts to virulent by single amino acid substitution to arginine at position 333 in glycoprotein. *Virus Res*. **119**, 208-215.

Takayama-Ito, M., Ito, N., Yamada, K., Sugiyama, M., and Minamoto, N. (2006a) Multiple amino acids in the glycoprotein of rabies virus are responsible for pathogenicity in adult mice. *Virus Res*. **115**, 169-175.

Tao, L., Ge, J., Wang, P., Zhai, H., Hua, T., *et al*. (2010) Molecular basis of Neurovirulence of Flury Rabies virus vaccine strains: Importance of the polymerase and the Glycoprotein R333Q mutation. *J. Virol.* **84**, 8926-8936.

Tarantola, A. (2017) Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, Its Prevention and Its Cure. *Trop. Med. Infect. Dis.* **2**, 5.

Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., *et al.* (2013) Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. *Science* **342**, 871-874.

Torres, C., Lema, C., Dohmen, F.G., Beltran, F., Novaro, L., *et al.* (2014) Phylodynamics of vampire bat-transmitted rabies in Argentina. *Mol. Ecol.* **23**, 2340-2352.

Troupin, C., Dacheux, L., Tanguy, M., Sabeta, C., Blanc, H., *et al.* (2016) Large-scale phylogenomic analysis reveals the complex evolutionary history of rabies virus in multiple carnivore hosts. *PLoS Pathog.* **12**, e1006041.

Tuffereau, C., Desmézières, E., Bénéjean, J., Jallet, C., Flamand, A., *et al.* (2001) Interaction of lyssaviruses with the low-affinity nerve-growth factor receptor $p75^{NTR}$. *J. Gen. Virol.* **82**, 2861-2867.

Turmelle, A.S., Allen, L.C., Schmidt-French, B.A., Jackson, F.R., Kunz, T.H., *et al.* (2010b) Response to vaccination with a commercial inactivated rabies vaccine in a captive colony of Brazilian free-tailed bats (*Tadarida brasiliensis*). *J. Zoo Wildl. Med*. **41**, 140-143.

Turmelle, A.S., Jackson, F.R., Green, D., McCracken, G.F., and Rupprecht, C.E. (2010a) Host immunity to repeated rabies virus infection in big brown bats. *J. Gen. Virol.* **91**, 2360-2366.

Tuttle, M. (2017) Rabies in Perspective. http://www.merlintuttle.com/resources-2/rabies-in-perspective/

Udow, S.J., Marrie, R.A. and Jackson, A.C. (2013) Clinical features of dog- and bat-acquired rabies in humans. *Clin. Infect. Dis*. **57**, 689-696.

Ugolini, G. (2011) Rabies virus as a transneuronal tracer of neuronal connections. *Adv. Imaging Electron Physics.* **79**, 165-202.

van Asch, B., Zhang, A.B., Oskarsson, M.C., Klütsch, C.F., Amorim, A., *et al.* (2013) Pre-Columbian origins of Native American dog breeds, with only limited replacement by European dogs, confirmed by mtDNA analysis. *Proc. Roy. Soc. Lond. Ser. B* **280**, 20131142.

van Eeden, C., Markotter, W., and Nel, L.H. (2011) Molecular phylogeny of Duvenhage virus. *S. Afr. J. Sci.* **107**, 1-5.

van Thiel, P.P.A., de Bie, R.M., Eftimov, F., Tepaske, R., Zaaijer, H.L., *et al*. (2009) Fatal human rabies due to Duvenhage virus from a bat in Kenya: failure of treatment with coma-induction, ketamine, and antiviral drugs. *PLoS Negl. Trop. Dis.* **3**, e428.

Vaughan, T.A. (1970) The muscular system. *In* W.A. Wimsatt (ed.), *Biology of Bats Volume 1*. London: Academic Press, pp.140-194.

Vázquez-Morón, S., Juste, J., Ibáñez, C., Ruiz-Villamor, E., Avellón, A., *et al*. (2008) Endemic circulation of European bat lyssavirus type 1 in serotine bats, Spain. *Emerg. Infect. Dis.* **14**, 1263.

Velasco-Villa, A., Mauldin, M.R., Shi, M., Escobar, L.E., Gallardo-Romero, N.F., *et al*. (2017) The history of rabies in the Western Hemisphere. *Antivir. Res*. **146**, 221-232.

Velasco-Villa, A., Orciari, L.A., Juárez-Islas, V., Gómez-Sierra, M., Padilla-Medina, I., *et al*. (2006) Molecular diversity of rabies viruses associated with bats in Mexico and other countries of the Americas. *J. Clin. Microbiol.* **44**, 1697-1710.

Viana, M., Mancy, R., Biek, R., Cleaveland, S., Cross, C., *et al.* (2014) Assembling evidence for identifying reservoirs of infection. *Trends Ecol. Evol*. **29**, 270-279.

Virgin, H.W., Wherry, E.J., and Ahmed, R. (2009) Redefining chronic viral infection. *Cell* **138**, 30-50.

Voigt, C.C. (2013) Bat flight with bad wings: is flight metabolism affected by damaged wings? *J. Exp. Biol*. **216**, 1516-1521.

Voigt-Heucke, S.L., Taborsky, M., and Dechmann, D.K. (2010) A dual function of echolocation: bats use echolocation calls to identify familiar and unfamiliar individuals. *Anim. Behav*. **80**, 59-67.

Vos, A., Kaipf, I., Denzinger, A., Fooks, A.R., Johnson, N., *et al*. (2007) European bat lyssaviruses – an ecological enigma. *Acta Chiropt*. **9**, 283-296.

Vos, A., Müller, T., Cox, J., Neubert, L., and Fooks, A.R. (2004b) Susceptibility of ferrets (*Mustela putorius furo*) to experimentally induced rabies with European bat lyssaviruses (EBLV). *J. Vet. Med*. **51B**, 55-60.

Vos, A., Müller, T., Neubert, L., Zurbriggen, A., Botteron, C., *et al*. (2004a) Rabies in red foxes (*Vulpes vulpes*) experimentally infected with European bat lyssavirus Type 1 (EBLV-1). *J. Vet. Med*. **51B**, 327-332.

Wacharapluesadee, S., Sintunawa, C., Kaewpom, T., Khongnomnan, K., Olival, K.J., *et al.* (2013) Group C betacoronavirus in bat guano fertilizer, Thailand. *Emerg. Infect. Dis.* **19**, 1349-1352.

Walker, P.J., Firth, C., Widen, S.G., Blasdell, K.R., Guzman, H., *et al*. (2012) Evolution of genome size and complexity in the Rhabdoviridae. *PLoS Pathog*. **11**, e1004664.

Wallace, R.M., Gilbert, A., Slate, D., Chipman, R., Singh, A., *et al*. (2014) Right place, wrong species: a 20-year review of rabies virus cross species transmission among terrestrial mammals in the United States. *PLoS One* **9**, e107539.

Wandeler, A. (2004) Epidemiology and ecology of fox rabies in Europe. *In* A.A. King, A.R. Fooks, M. Aubert and A.I. Wandeler (eds.), *Historical perspective of Rabies in Europe and the Mediterranean Basin*. Paris, France: OIE. pp. 201-213.

Wang, L., Tang, Q., and Liang, G. (2014) Rabies and rabies virus in wildlife in mainland China, 1990–2013. *Int. J. Infect. Dis.* **25**, 122-129.

Wang, Z.W., Sarmento, L., Wang, Y., Li, X.Q., Dhingra, V., *et al.* (2005) Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. *J. Virol.* **79**, 12554-12565.

Warrell, M., and Warrell, D. (2004) Rabies and other lyssavirus diseases. *Lancet* **363**, 1907.

Warrell, M.J. (2009) Rabies and Other Lyssavirus Infections. *In* A.J. Zuckerman, J.E. Banatvala, B.D. Schoub, P.D. Griffiths and P. Mortimer (eds.), *Principles and Practice of Clinical Virology, Sixth Edition*. Chichester: Wiley, pp.777-806.

Weaver, K.N., Alfano, S.E., Kronquist, A.R., and Reeder, D.M. (2009) Healing rates of wing punch wounds in free-ranging little brown myotis (*Myotis lucifugus*). *Acta Chiropt*. **11**, 220-223.

Weir, D., Annand, E., Reid, and Broder, C. (2014) Recent observations on Australian bat lyssavirus tropism and viral entry. *Viruses* **6**, 909-926.

Wekerle, H. (2006) Breaking ignorance: the case of the brain. *Curr. Top. Microbiol. Immunol*.

Wellenberg, G.J., Audry, L., Ronsholt, L., van der Poel, W.H., Bruschke, C.J., *et al*. (2002) Presence of European bat lyssavirus RNAs in apparently healthy *Rousettus aegyptiacus* bats. *Arch. Virol*. **147**, 349-361.

Wertheim, J.O., and Pond, S.L.K. (2011) Purifying selection can obscure the ancient age of viral lineages. *Mol. Biol. Evol*. **28**, 3355-3365.

Wertheim, J.O., Chu, D.K., Peiris, J.S., Pond, S.L.K., and Poon, L.L. (2013) A case for the ancient origin of coronaviruses. *J. Virol*. **87**, 7039-7045.

Wertheim, J.O., Smith, M.D., Smith, D.M., Scheffler, K., and Pond, S.L.K. (2014) Evolutionary Origins of Human Herpes Simplex Viruses 1 and 2. *Mol. Biol. Evol.* **31**, 2356-2364.

WHO (2007) Oral Vaccination of Dogs Against Rabies - Guidance for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies.

Widmaier, E.P., Harmer, T.L., Sulak, A.M., and Kunz, T.H. (1994) Further characterization of the pituitary-adrenocortical responses to stress in Chiroptera. *J. Exp. Zool.* **269A**, 442-449.

Wilkinson, G.S., Carter, G.G., Bohn, K.M., and Adams, D.M. (2016) Non-kin cooperation in bats. *Phil. Trans. Roy. Soc. Lond. Ser. B* **371**, 20150095.

Williams, J. E., Imlarp, S., Top, F., Cavanaugh, D., and Russell, (1976) Kaeng Khoi virus from naturally infected bedbugs (Cimicidae) and immature free-tailed bats. *Bull. WHO* **53**, 365-369.

Williams, J.E., Imlarp, S., Top, F.H., Cavanaugh, D., and Russell, P.K. (1976) Kaeng Khoi virus from naturally infected bedbugs (Cimicidae) and immature free-tailed bats. *Bull. WHO* 53, 365-9.

Winkler, W.G. (1968) Airborne rabies virus isolation. *Bull. Wildl. Dis. Assoc.* **4**, 37-40.

Winkler, W.G., Baker, E.F., and Hopkins, C.C. (1972) An outbreak of non-bite transmitted rabies in a laboratory animal colony. *Amer. J. Epidem.* **95**, 267-278.

Wolfe, N.D., Dunavan, C.P., and Diamond, J. (2007) Origins of major human infectious diseases. *Nature* **447**, 279-283.

Woolhouse, M.E., Scott, F., Hudson, Z., Howey, R., and Chase-Topping, M. (2012) Human viruses: Discovery and emergence. *Phil. Trans. Roy. Soc. Lond. Ser. B* **367**, 2864-2871.

Wright, E., Hayman, D.T., Vaughan, A., Temperton, N.J., Wood, J.L., *et al.* (2010) Virus neutralising activity of African fruit bat (*Eidolon helvum*) sera against emerging lyssaviruses. *Virology* **408**, 183-189.

Wu, P., Hu, R., Zhang, Y., Dong, G., and Rupprecht, C.E. (2009) Reemerging Rabies and lack of systemic surveillance in People's Republic of China. *Emerg. Infect. Dis.* **15**, 1159-1164.

Wu, Z., Yang, L., Ren, P., He, G., Zhang, J., *et al.* (2015) Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases (and supplementary information, tables and figures). *ISME Journal* **10**, 609-620.

Wunner, W.H. (2007) Rabies virus. *In* A.C. Jackson and W.H. Wunner (eds.) *Rabies* (2nd edition). London: Academic Press, pp. 23-68.

Zepeda-Mendoza, M.L., Xiong, Z., Escalera-Zamudio, M., Runge, A.K., Thézé, J., *et al.* (2018) Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat. *Nature Ecol. Evol.*

Zerboni, L., and Arvin, A.M. (2016) The Pathogenesis of Varicella-Zoster Virus Neurotropism and Infection. *In* C.S. Reiss (ed.), *Neurotropic Viral Infections*. *Volume 2: Neurotropic Retroviruses, DNA Viruses, Immunity and Transmission*. Springer, pp. 135-173.

Zhang, D., He, F., Bi, S., Guo, H., Zhang, B., *et al*. (2016) Genome-wide transcriptional profiling reveals two distinct outcomes in central nervous system infections of rabies virus. *Front. Microbiol.* **7**, 751.

Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K.A., *et al.* (2012) Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460.

Zhang, Y.Z., Fu, Z.F., Wang, D.M., Zhou, J.Z., Wang, Z.X., *et al.* (2008) Investigation of the role of healthy dogs as potential carriers of rabies virus. *Vect.-Borne Zoon. Dis.* **8**, 313-320.

Zhou, M., Zhou, Z., Kia, G.S.N., Gnanadurai, C.W., Leyson, C.M., *et al*. (2013). Complete Genome Sequence of a Street Rabies Virus Isolated from a Dog in Nigeria. *Genome Announcements* **1**, e00214–12.

Zieger, U., Cheetham, S., Santana, S. E., Leiser-Miller, L., Matthew-Belmar, V., *et al*. (2017) Natural exposure of bats in Grenada to rabies virus. *Infect. Ecol. Epidemiol.* **7**, 1332935.

Zieger, U., Marston, D.A., Sharma, R., Chikweto, A., Tiwari, K., *et al*. (2014) The phylogeography of rabies in Grenada, West Indies, and implications for control. *PLoS Negl. Trop. Dis.* **8**, e3251.