The impact of novel lyssavirus discovery

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**The global discovery of novel lyssaviruses is of continued
scientific interest through its importance to both public and
animal health. Lyssaviruses cause an invariably fatal en-
cephalitis that is more commonly known as rabies. The
term rabies has a long history in human society, as rabies
virus (RABV) is the only pathogen that is associated with
100% fatality once the onset of clinical disease has started.
Although predominantly associated across the globe with
domestic and feral dog populations, the association of
bats is clear. Whilst evolutionarily associated with bats,
RABV is most commonly transmitted to human populations
through the bite of an infected dog and dogs are considered
the primary reservoir of disease. Indeed, RABV does cause
more than an estimated 70 000 deaths every year globally
in human populations and whilst this is largely in areas
where the disease is endemic, areas that remain free of
rabies must remain vigilant to the risk of re-incursion of
disease. Characterisation of novel lyssaviruses is of impor-
tance on several levels. Not least to investigate the patho-
genesis and potential transmission routes of different
lyssavirus species but also to assess the potential effect of
post-exposure treatments and vaccination should human
exposure occur. Bat lyssaviruses and the problems associ-
ated with novel discoveries and the potential impact they
have on both human and animal populations are discussed.**

The global discovery of lyssaviruses is of continued scientific
interest through its importance to both public and animal health.
Lyssaviruses cause an invariably fatal encephalitis referred to as
rabies. The term rabies has a long history in human society, as rabies
virus (RABV) is the only pathogen that is associated with 100%
fatality once the onset of clinical disease has started[1]. Whilst
predominantly associated across the globe with domestic and feral

dog populations, the association of bats with lyssaviruses is clear.
Historically, rabies has a long association with hematophagous bats
across Central and South America and this association, with
'mysterious blood feeding creatures of the night', has cemented
rabies into the conscience of human populations. Alongside this
the more typical association of the virus with aggressive dogs, the
horrific clinical disease seen in human infection, and the invariably
fatal nature of infection, has led to rabies being the most feared
pathogen known to man. Such clinical manifestations have held the
imagination of humanity since the earliest reports of canine and
human madness. Whilst evolutionarily associated with bats, rabies
virus is most commonly transmitted to human populations through
the bite of an infected dog. Indeed, RABV does cause thousands of
deaths every year globally in human populations and whilst this is
largely in areas where the virus is endemic, areas that remain free of
rabies must remain vigilant to the risk of re-incursion of disease[2].
Alongside the burden seen from dogs, wildlife species also play an
important role in the epidemiology of disease although the paucity
of data on wild animal populations, their distribution and the
generally sporadic interactions between different wildlife popula-
tions and domesticated carnivore species means that the role of
wildlife and the epidemiology of the virus is often unclear.

Alongside RABV strains that are predominantly associated with
terrestrial carnivore species, a number of other genetically and to
some extent antigenically related viruses exist within the lyssavirus
genus[3]. Currently, 14 viruses, in addition to RABV are associated
with bats and are classified within the genus[4], while few are well
characterised from either an epidemiological or clinical disease
perspective. Three bat-associated viruses, Lleida bat lyssavirus,
Gannoruwa bat lyssavirus and Taiwan bat lyssavirus, are not offi-
cially classified within the *Lyssavirus* genus, and remain as tentative
species only. Although RABV causes a significant annual disease

Table 1. The association of lyssavirus species with bats. For species coloured green there appears to be no protection afforded by current rabies vaccines. IKOV and MOKV are absent as there has been no association with bat species.

Continent	Geographical distribution of isolates	Lyssavirus species	Bat species associated with lyssavirus infection	Common bat name	Human fatalities reported
The Americas	North and South America	Rabies lyssavirus (RABV)[A]	*Eptesicus fuscus*	Big brown bat	Yes
			Tadarida brasiliensis	Mexican/Brazilian free-tail bat	
			Lasionycteris noctivagens	Silver-haired bat	
			Perimyotis subflavus	Tri-coloured bat	
			Desmodus rotundus	Vampire bat	
Africa	Nigeria, Senegal, Ghana, Kenya	Lagos bat lyssavirus (LBV)	*Eidolon helvum*	Straw coloured fruit bat	No
	France (ex-Togo or Egypt), Kenya,		*Rousettus aegyptiacus*	Egyptian fruit bat	
	Central African Republic		*Micropteropus pussilus*	Dwarf epaulet fruit bat	
	Ghana		*Epomorphus giambianus*	Gambian epauletted fruit bat	
	Ghana		*Epomops buettikoferi*	Buettikofer's epauletted fruit bat	
	Guinea		*Nycteris gambiensis*	Gambian slit-faced bat	
	South Africa		*Epomorphorus wahlbergi*	Wahlberg's epauletted fruit bat	
	Kenya	Shimoni bat lyssavirus (SHIBV)	*Hipposideros commersoni*	Commerson's leaf-nosed bat	No
	South Africa, Kenya[B]	Duvenhage lyssavirus (DUVV)	*Miniopterus sp.*	Undefined	Yes
	Zimbabwe		*Nycteris thebaica*	Egyptian slit-faced bat	
Europe	France, Germany, Spain	European bat 1 Lyssavirus (EBLV-1)	*Eptesicus serotinus*	Serotine bat	Yes
	The Netherlands, Switzerland, UK, France, Germany, Luxembourg, Finland	European bat 2 lyssavirus (EBLV-2)	*Myotis daubentonii*	Daubenton's bat	Yes
	Germany, France	Bokeloh bat lyssavirus (BBLV)	*Myotis nattereri*	Natterer's bat	No
	Spain	Lleida bat lyssavirus (LLEBV)[C]	*Miniopterus schreibersii*	Common bent-winged bat	No
Eurasia	Kyrgystan	Aravan lyssavirus (ARAV)	*Myotis blythi*	Lesser mouse-eared bat	No
	Russian Federation, China	Irkut lyssavirus (IRKV)	*Murina leucogaster*	Greater tube-nosed bat	Yes
	Tajikistan	Khujand lyssavirus (KHUV)	*Myotis mystacinus*	Whiskered bat	No
	Russian Federation, Kenya[D]	West Caucasian bat lyssavirus (WCBV)	*Miniopterus schreibersii*	Common bent-winged bat	No
Australasia	Australia	Australian bat lyssavirus (ABLV)	*Pteropus alecto*	Black flying fox and related sp.	Yes
			Saccolaimus flaviventris	Yellow-bellied sheath-tailed bat	
Asia	Sri Lanka	Gannoruwa bat lyssavirus (GBLV)[C]	*Pteropus medius*	Indian flying fox	No
	Taiwan	Taiwan bat lyssavirus (TWBLV)[C]	*Pipistrellus abramus*	Japanese house bat	No

[A]More than 50 bat species have been implicated in RABV infection across the Americas- the most frequently reported species are listed for convenience.
[B]Case reported from The Netherlands but origin of exposure was Kenya.
[C]Not yet classified as lyssaviruses by the International Committee for the Taxonomy of Viruses (ICTV).
[D]Serological evidence of infection in Kenya.

burden to human populations globally, the association of these other lyssaviruses with human fatalities is poorly characterised (Table 1). This may be because there are genuinely fewer cases of infection with these viruses across human and animal populations or, conversely may reflect the inability of the most commonly used diagnostic tool, the antigen detection based Fluorescent Antibody Test (FAT), to differentiate between lyssavirus species and a lack of a secondary confirmatory test in endemic regions that can genetically type virus[5]. This feature is important and as more divergent lyssaviruses are discovered the ability of commercial conjugates to detect them requires assessment. These factors mean that the true epidemiological situation regarding these lyssaviruses remains unclear and consequently the burden of disease to animal and human health remains undefined[6].

Divergent lyssaviruses have been recognised for over 65 years. Initial discoveries of viruses causing rabies disease originated in the Old World in the 1950s where serological profiling using monoclonal antibodies characterised viruses similar to RABV[7]. A steady flow of virus discovery then continued until molecular methods were developed that superseded antibody based classification of new pathogens. Currently, sequencing technologies are able to rapidly type viruses genetically and as such lyssaviruses continue to be discovered[8,9]. Apart from those already detected and partially characterised, novel species continue to be detected that then require characterisation and classification. The importance of novel lyssaviruses remains unknown but the potential for fatal infection following spill over events dictates that some importance must be placed on their virological characterisation. This has become increasingly important with the heightened interest in bats as biological entities and as reservoirs of zoonotic pathogens that have crossed species barriers to drive large mortality events in human and animal populations. Furthermore, the potential for interactions with bat species has increased with the encroachment of human populations into areas of forestation and the popularity of human activities such as caving and potholing[10].

The association of lyssaviruses with bats provides several interesting conundrums regarding the epidemiology of these viruses[11]. RABV is present across terrestrial carnivore populations across the globe but is only associated with the infection of insectivorous, hematophagous and to a lesser extent frugivorous bats across the Americas. In the Americas, following the successful elimination of rabies from domestic carnivore species and, following extensive oral vaccination programs, reduction of disease within wildlife populations, bats represent an ever present remaining source of virus for potential infection and spill over transmission events that continue to cause fatalities in the human populations. Indeed,

recent reports have highlighted the importance of seeking post-exposure prophylaxis wherever interaction with a potentially rabid bat has occurred, even in the absence of any actual contact between the animal and the human individual in question[12]. Interestingly however, the detection of classical RABV has never been reported across the 'Old World' in bats[13]. In contrast, the other viruses that are classified within the lyssavirus genus have, in the main, been isolated from different bat species across the Old World with a complete lack of reporting of any lyssavirus species, apart from RABV, in the Americas (Figure 1). The basis for this apparent division remains unknown. It is probable that RABV has existed in blood-sucking and insectivorous bats in the Americas for millennia. With the introduction of European dog rabies and large agricultural hosts (cattle, horses etc.) following the European colonisation of the Americas, the incidence of RABV in these new hosts likely increased exponentially. Further, increased agricultural prey species likely impacted significantly to the increase in vampire bat populations.

A key factor that defines the relationship between viruses and their hosts is the ability to be transmitted and maintained within a population. The maintenance of lyssaviruses within bat populations is poorly understood. Whilst bats can be clearly identified as reservoir hosts for lyssaviruses, where associations have been defined, they do not fulfil the classic ideal of a symbiotic relationship between microbe and host. In this sense, the ability of viruses to cause clinical disease is of interest. For numerous other pathogens for which bats are considered reservoirs, including high profile viral zoonotic pathogens such as Ebola virus and Nipah virus, infection generally occurs in the absence of clinical disease in the bat. For lyssaviruses, although our understanding may be limited, this feature of virus host interactions is not true. Indeed, lyssaviruses do cause clinical disease in the bats they infect and in the vast majority of cases it is only through the observation of clinical manifestations of disease that these viruses are discovered. This is a feature not restricted to bat infection, but one that covers lyssavirus infection of all mammals. However, recent evidence has suggested that mammals can be exposed to virus, mount an immunologically detectable response (generally a humoral response) and clear the virus. Both in bat populations where serological positivity can range significantly across a roost and human populations, evidence of exposure in the absence of clinical disease has been reported. What however constitutes exposure against an interaction that leads to productive infection and disease is ill defined, as are the mechanisms that dictate the outcome of any potential exposure/infection event. Assessment of innate signalling mechanisms in bats, and other species, following exposure needs to be completed to understand the mechanisms that drive infection.

Another key question when considering bat lyssavirus infection is the potential for cross species transmission events (CSTs)[14]. Whilst few CSTs have been reported where the bat variant has been maintained in terrestrial hosts[15,16], the potential for such events remains. Certainly across the Old World, where non-rabies lyssaviruses appear to predominate in bat populations whilst RABV appears to circulate terrestrially, such events may occur more often but without any evidence for viral adaptation and transmission. Current diagnostic methods to genetically type rabies virus at post mortem cannot differentiate between lyssaviruses and as such CST events may be missed. Certainly, without the adoption of molecular tools to genetically type circulating virus variants such events will remain undefined.

A final element of importance when considering the detection of novel lyssaviruses, and their effect on human and animal populations, is the role of neutralising antibodies. Whilst nearly 100% fatal following the development of clinical disease, it has long been established that a neutralising antibody titre over a defined

threshold will protect individuals from the development of disease[17]. However, the cut-off for a serological neutralising antibody titre is poorly defined for numerous viruses within the continually expanding lyssavirus genus and as such the discovery of novel viruses requires investigation as to the efficacy of existing pre- and post-exposure preparations[6,18–23]. Of the more divergent viruses, there appears to be no vaccine afforded protection whilst the less divergent may be neutralised by a vaccinal response[5,6,24,25].

The relative impact of these viruses on human populations is low, as evidenced by the low number of human fatalities associated with these viruses in countries that are free of terrestrial rabies where diagnostic capabilities are able to thoroughly investigate cases of encephalitis. Maintenance of such rabies free areas is important and the OIE definition of what constitutes freedom from rabies disease is clear and valid. Numerous island nations, including the United Kingdom and Australia, are defined by the OIE as 'rabies free', because rabies virus is absent in terrestrial mammals. However, both serological and virological evidence for
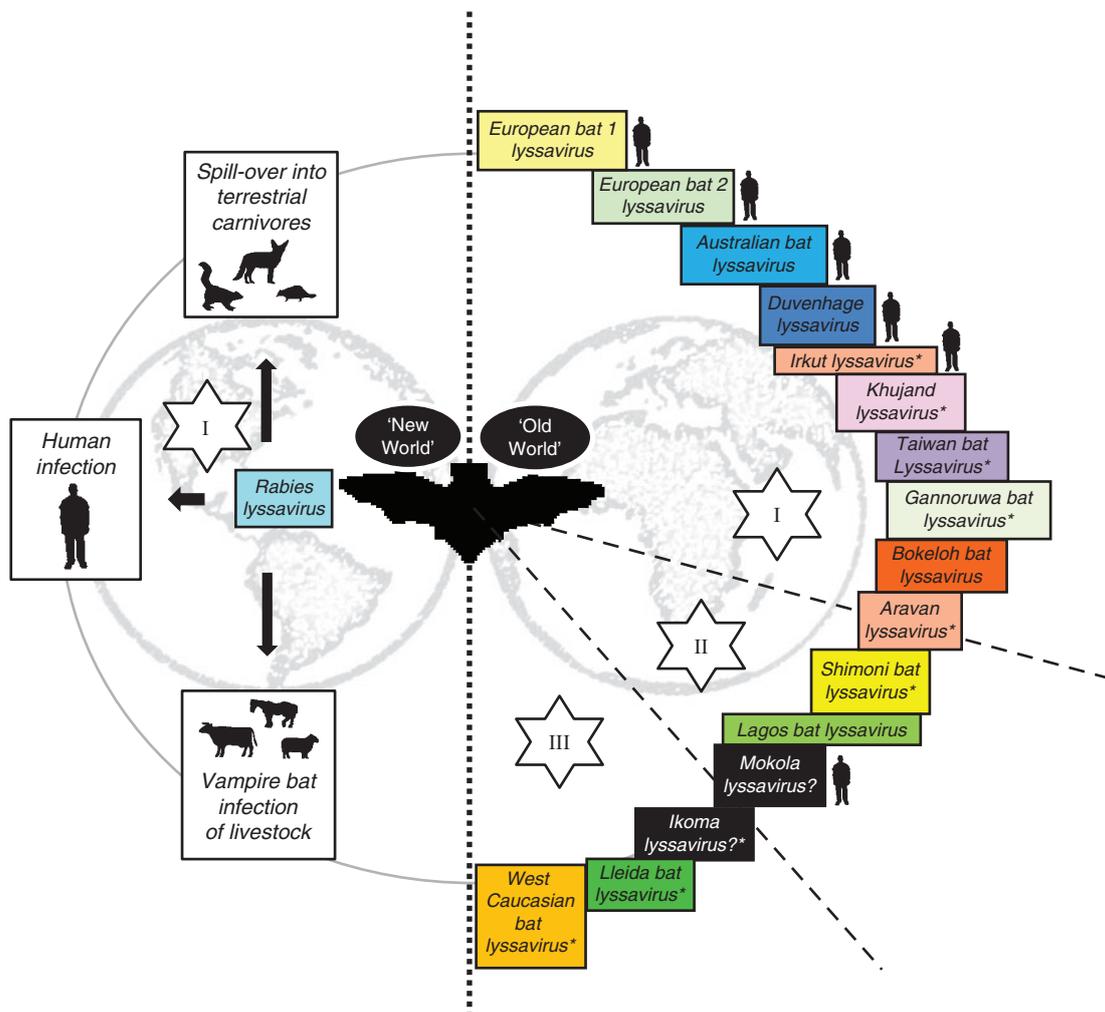
Figure 1. The global diversity of the bat lyssaviruses. Where viruses have been associated with human fatalities the species is flanked by a human silhouette. The division of lyssavirus species into antigenically distinct phylogroups is shown as roman numerals within stars. *Denotes where only a single isolate exists. Lyssavirus species shown as black boxes have not been reported in bat species.

lyssaviruses continues to increase across bat populations. As such, an increased knowledge of the mechanisms behind how these viruses persist, and how they cross the species barrier will be critical in ensuring that risk assessments surrounding human and animal health are optimised.

Acknowledgements

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Biographies

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