Epidemiol. Infect. (2003), **131**, 1029–1039. © 2003 Cambridge University Press
DOI: 10.1017/S0950268803001481 Printed in the United Kingdom

REVIEW ARTICLE
European bat lyssaviruses: an emerging zoonosis

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This review is dedicated to the late Dr Arthur King (CVL, Weybridge, UK) for his outstanding contribution to rabies research.

(*Accepted 21 August 2003*)

SUMMARY

In Europe, two bat lyssaviruses referred to as European bat lyssaviruses (EBLVs) types 1 and 2 (genotypes 5 and 6 respectively) which are closely related to classical rabies virus are responsible for an emerging zoonosis. EBLVs are host restricted to bats, and have been known to infect not only their primary hosts but also in rare circumstances, induce spillover infections to terrestrial mammals including domestic livestock, wildlife and man. Although spillover infections have occurred, there has been no evidence that the virus adapted to a new host. Since 1977, four human deaths from EBLVs have been reported. None of them had a record of prophylactic rabies immunization. Only fragmentary data exist about the effectiveness of current vaccines in cross-protection against EBLVs. It is clear that EBLV in bats cannot be eliminated using conventional strategies similar to the control programmes based on vaccine baits used for fox rabies in Europe during the 1980s. Due to the protected status of bats in Europe, our knowledge of EBLV prevalence and epidemiology is limited. It is possible that EBLV is under-reported and that the recorded cases of EBLV represent only a small proportion of the actual number of infected bats. For this reason, any interaction between man and bats in Europe must be considered as a possible exposure. Human exposure through biting incidents, especially unprovoked attacks, should be treated immediately with rabies post-exposure treatment and the bat, where possible, retained for laboratory analysis. Preventative measures include educating all bat handlers of the risks posed by rabies-infected animals and advising them to be immunized. This review provides a brief history of EBLVs, their distribution in host species and the public health risks.

INTRODUCTION

Rabies is a statutory notifiable exotic disease in both animals and man that represents such a threat to animal and human health that control measures aimed at preventing its establishment are subject to specific legislation. Classical rabies virus (RABV) is one of seven lyssaviruses (see Table 1); all are capable of causing clinically indistinguishable fatal disease in humans and other mammals. In Europe, two lyssaviruses, referred to as European bat lyssaviruses types 1 and 2 (EBLV-1 and EBLV-2), pose a threat to any human who comes into contact with an infected bat. However, infections caused by EBLVs in man are

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Table 1. *Classification of the* Lyssavirus *genus*

Genotye	Virus name	Distribution and source species	Other known susceptible hosts
1 (RABV)	Classical rabies virus (several different host-adapted strains)	Carnivores almost world-wide Bats in the Americas	Wide range of mammals
2 (LBV)	Lagos bat virus	Fruit bats in Africa	Dogs and cats
3 (MOKV)	Mokola virus	Africa. No reported bat association	Shrews, rodents, dogs, cats and humans
4 (DUVV)	Duvenhage virus	Insectivorous bats in southern Africa	Humans
5 (EBLV-1)	European bat lyssavirus type 1	Insectivorous bats (especially *Eptesicus serotinus*) in Europe	Humans (Ukraine and Russia) sheep (Denmark), stone marten (Germany)
6 (EBLV-2)	European bat lyssavirus type 2	Insectivorous bats (especially *Myotis* species) in Europe, Kyrghyzstan and Tajikstan	Humans (UK and Finland)
7 (ABLV)	Australian bat lyssavirus	Insectivorous and fruit bats in eastern Australia	Humans (Australia)
Proposed genotypes	Aravan and Khujand	Insectivorous bats (*Myotis* species) in Kyrghyzstan and Tajikstan	None known

rarely reported [1–5]. Human disease caused by other lyssaviruses: Mokola virus, Duvenhage virus and Australian bat lyssavirus are also rare [6–8].

EBLVs are closely related to RABV and have been known to infect not only their primary hosts (insectivorous bats) but also on occasion other incidental animal hosts [9, 10].

Effective surveillance is a vital component of any control policy intended to maintain the rabies-free (virus/disease) status of a specific country. European countries that are currently free of indigenous rabies in terrestrial mammals (i.e. with no new indigenous case being reported for a period of 2 years) are considered to be rabies free. Currently the Office International des Epizooties (OIE) excludes bat rabies cases when declaring a country rabies free. This definition may, however, change in the future.

In specific European countries, EBLV infection is currently monitored by passive surveillance of dead or ill bats, using a variety of detection methods. Furthermore, if the rabies-free status of any European country is to be maintained then susceptible terrestrial animals must be shown to be both disease and exposure free.

During the 1920s, RABV was identified in insectivorous bats in Brazil and in the 1930s in frugivorous and insectivorous bats in Trinidad. However, it was not until a young boy in Florida, USA was bitten on the chest by a RABV-infected insectivorous bat in 1953 that interest in rabies in bats intensified. In Europe, rabies-like disease has been diagnosed in bats since 1954 [11]. However, unlike the viruses that infect American bat species, which are classified as variants of classical RABV (genotype 1), serology, genetic typing and cross-protection studies have demonstrated that the viruses of European bats are distinct [12–15].

In Europe EBLV infections in bats have been confirmed, mainly in The Netherlands, Germany, Denmark and Poland [16–18]. In addition, low levels of EBLV cross-species transmission events have been reported in Europe. To date, most ($>95\%$) viral isolates have been of EBLV type 1 and are predominantly associated with the serotine bat (*Eptesicus serotinus*), whereas EBLV type 2 appears to be associated with *Myotis* species [19]. As a result of recent serological studies in Spanish bat colonies it has been suggested that EBLV-1 is more prevalent than originally suspected and that its host range among bat species is diverse [20]. In contrast, the numbers of confirmed EBLV-2 cases in bats have remained low.

Bats are the only mammals that can fly and are distributed widely with more than 1100 species reported throughout the world. Bats are, however, still persecuted by man and in many areas of the world they are becoming endangered species. On a global scale, approximately 25% of bat species are 'threatened', with a further 25% 'near threatened' and likely to become threatened unless careful consideration is given to their status. Although the remaining 50% of bat species are not in danger, some species are considered threatened at a local level, especially in parts of Europe. They are afforded legal protection throughout Europe and are listed as 'European protected species' [21].

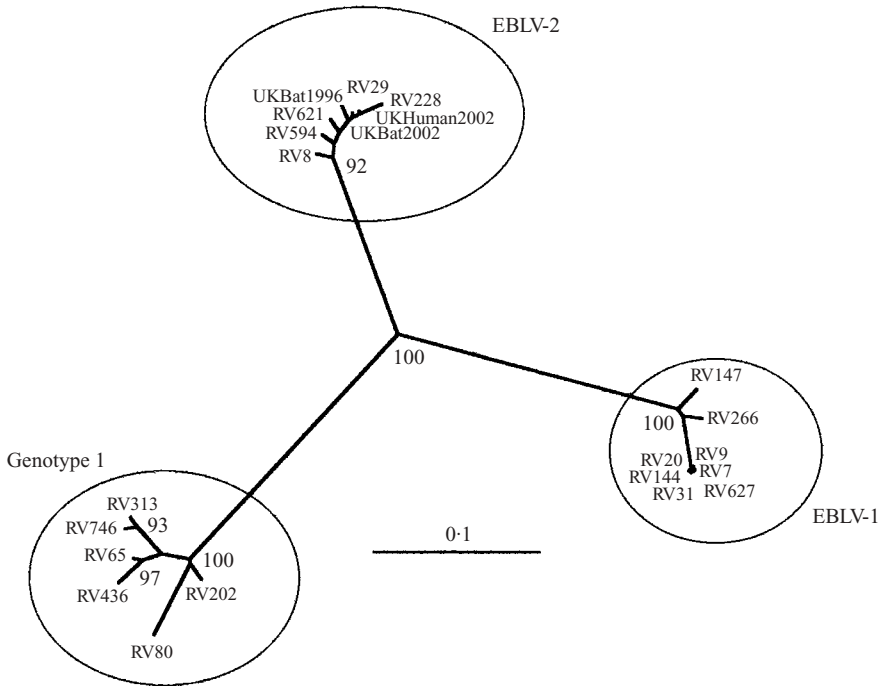

Fig. Radial tree phylogenetic analysis of viral isolates from genotypes 1, 5 and 6 using a 400 bp sequence of the *Lyssavirus* nucleoprotein coding sequence. Significant bootstrap values >70 are included.

Whilst strategies are converging for the control of rabies in domestic animals and sylvatic reservoirs, the threat of a 'spillover' infection from a bat to terrestrial mammals including man still exists, indicating that EBLVs pose a risk to public health.

TAXONOMY

Lyssavirus genus

The genus *Lyssavirus* is one of four genera that form the family Rhabdoviridae within the order Mononegavirales [22]. The *Lyssavirus* genus comprises classical RABV (genotype 1) and six genetically divergent strains [23] that discriminate the members of this genus (Table 1). EBLV-1 (genotype 5) and EBLV-2 (genotype 6) are restricted to insectivorous bats in Europe. Lagos bat virus (LBV; genotype 2), Mokola virus (MOKV; genotype 3) and Duvenhage virus (DUVV; genotype 4) are restricted geographically to Africa. LBV is the only lyssavirus that has never been reported to infect man. Human fatalities caused by MOKV, DUVV and Australian bat lyssavirus (ABLV; genotype 7) [24] are rare. All seven genotypes, with one exception (MOKV), have been isolated from bats. Recently, two additional viruses isolated from bats in the former Soviet Union (Aravan and Khujand virus) have been proposed as new members of the *Lyssavirus* genus [25].

Serotype classification of EBLVs

Initially EBLV-1 isolates from serotine bats were characterized with monoclonal antibodies, placing them in serotype 4 as a Duvenhage-like virus [13, 26–28]. A second group of bat viruses was identified in *Myotis dasycneme* and *Myotis daubentonii* [29]. By 1990 they were characterized as two distinct biotypes EBLV-1 and EBLV-2 (genotypes 5 and 6) [14, 26]. Prior to the wider availability of genetic analysis, rabies and other lyssaviruses were identified by serotype using panels of anti-nucleocapsid monoclonal antibodies (Mab-Ns) [30–32]. These panels were able to differentiate between serotypes 1 (RABV and ABLV), 2 (LBV), 3 (MOKV), 4 (DUVV) and representatives of the EBLV-1 (genotype 5) from Denmark and EBLV-2 (genotype 6) from Finland.

Genotypic classification of EBLV-1 and EBLV-2

EBLV-1 (types 1a and 1b; genotype 5) and EBLV-2 (types 2a and 2b; genotype 6) are genetically and antigenically related to RABV. Both EBLVs, are however, significantly different from each other (Fig.; Table 2).

Table 2. *Isolates used for the comparative analysis*

Identification number	Genotype	Country of isolation	Animal
RV65	1	Poland	Unknown
RV80	1	Czech Republic	Unknown
RV202	1	Turkey	Dog
RV313	1	Germany	Fox
RV436	1	Estonia	Dog
RV746	1	France	Fox
RV7	5 [1a]	Denmark	Bat
RV9	5 [1a]	Germany	Bat
RV20	5 [1a]	Denmark	Bat
RV31	5 [1a]	The Netherlands	Bat
RV147	5 [1b]	Germany	Bat
RV144	5 [1a]	Germany	Bat
RV266	5 [1b]	France	Bat
RV627	5 [1a]	Denmark	Sheep
RV8	6 [2b]	Finland	Human
RV29	6 [2a]	The Netherlands	Bat
RV228	6 [2a]	The Netherlands	Bat
RV594	6 [2a]	Switzerland	Bat
RV621	6 [2b]	Switzerland	Bat
RV628	6 [2a]	UK 1996	Bat
RV1332	6 [2a]	UK 2002	Bat
RV1333	6 [2a]	UK 2002	Human

Although the viruses are separated geographically, evidence suggests a common evolutionary path between the EBLVs and DUVV [14, 15].

Genetic analyses of these bat lyssaviruses began in 1992 based on the whole and partial N gene sequences [14, 33–36]. They allowed the genetic classification of lyssaviruses to expand from 4 to 6. Kissi and coworkers [36] have shown that there is a low heterogeneity between EBLVs and classical RABV N gene (3·3%). A more comprehensive study was carried out by Amengual and others in 1997 [19]. Forty-seven EBLV isolates plus two African lyssaviruses (DUVV viruses) associated with insectivorous bats were compared at the molecular level in order to evaluate their evolutionary relationships. The phylogenetic study was based on the 3′ partial N gene sequence. Both nucleotide and amino-acid sequences placed the viruses into three separate clusters, namely their respective genotypes: genotype 4 (DUVV), genotype 5 (EBLV-1) and genotype 6 (EBLV-2). The complete glycoprotein sequences of EBLV-1 and -2 have been published recently and compared to representatives of the other five genotypes [37].

The main outcome has been the proposal of two *Lyssavirus* phylogroups, one containing genotypes 1, 4–7 and the other containing genotypes 2 and 3; this suggests possible evolutionary links that are common to each phylogroup [15, 19].

EPIDEMIOLOGY

Evolution of EBLVs

Phylogenetic studies have established similarities between RABV strains and related these to the species of bat that they infect [31, 38–40]. In Europe, for reasons unknown, the virus responsible for classical RABV (genotype 1) has not been detected within native bat species. However, two related viruses fill this ecological niche, namely EBLV-1 and -2.

Prevalence of EBLVs in bats from Europe

Although they were undoubtedly not the first European bats to be infected with rabies, during 1954–1984 fifteen cases of rabies in bats were reported from Germany (7), Yugoslavia (3), Ukraine (2), Turkey (1), Greece (1) and Poland (1). Between 1985 and 2002, that total increased to greater than 673, and cases were found more widely throughout Europe – in The Netherlands, Denmark, Germany, Poland, France, Spain, Switzerland, Ukraine, Czechoslovakia, Slovak Republic, Hungary and the United Kingdom [41, 42]. EBLV-1 appears to be the more prevalent virus, infecting over 95% of all EBLV-positive bats recorded between 1977 and 2000 [17, 43]. In the 3-year period 1999–2001, 25 181 cases of animal rabies have been reported in Europe, of which 110 (0·44%) were in bats.

EBLV-1

It is speculated that the different EBLV-1 lineages were introduced into parts of northern Europe from two directions, EBLV-1a being the most recently introduced strain from a North African origin via the south of Spain. EBLV-1a exhibits a west–east European division whilst EBLV-1b has a north–south distribution. All isolates were from *E. serotinus* with the exception of one from *Vespertilio murinus* from the Ukraine (1993). EBLV-1a and -1b could then represent two variant groups adapted to the same host. The Netherlands is the only country from which both EBLV-1a and -1b have been isolated. In 1997 an EBLV-1a was also found in frugivorous bats in a zoo in Denmark. This isolate was identified using sequence analyses of the N (partial) and G genes [44].

Spillover cases in terrestrial animals including zoo bats

EBLV-1 has been isolated from three sources other than indigenous European bats. These were captive (zoo) fruit bats in Denmark during 1997–1998, sheep in Denmark during 1998–2002 and a stone marten in Germany during 2001. There have been no reported spillover cases of EBLV-2 in animals.

Zoo bats

There is an increasing body of evidence to suggest that bats tolerate EBLV infection. EBLV-1 was isolated from captive colonies of Egyptian fruit bats (*Rousettus aegyptiacus*) in zoos in Western Europe. EBLV-1a was reported in *R. aeygptiacus* imported from The Netherlands (Rotterdam Zoo) to establish a colony in Odense Zoo, Denmark [45]. Throughout this event, however, no bat died of rabies or showed symptoms. There was no clinical outbreak in the bat colony, however, suggesting that EBLV-1 elicited a clinically silent infection. This case appears to be unique, and has a number of controversial aspects [46].

Van der Poel et al. [44] used bat and mouse inoculation tests to suggest that *R. aegyptiacus* could be victims of lyssavirus besides acting as reservoir hosts of infection. Wellenbourg et al. [47] estimated the non-lethal presence of EBLV-1a to be in up to 85% of apparently healthy animals in a captive colony in Rotterdam from tests on 40 bats from Rotterdam and 3 from Odense.

In a recent study, the EBLV-1 genome was detected in a range of tissues from apparently healthy specimens of both Schreiber's bent-winged bat (*Miniopterus schreibersii*) and the greater horseshoe bat (*Rhinolophus ferrumequinum*) [20]. These studies also demonstrated, by repeat humane blood sampling of selected bat colonies, that seropositive individuals could be detected over a 6-year time period. This illustrates that bats may survive EBLV infection with possible long-term maintenance of the virus in infected healthy individuals.

Sheep (*Ovis aries*) in Denmark

In 1998, EBLV-1a was isolated from individual sheep in three flocks from Denmark. This was the first incidence of an EBLV spillover into terrestrial mammals. In April 2002, one further instance in sheep, also in Denmark, was reported [10]. Bats have been recognized to share the same dwellings as domestic livestock and it is therefore conceivable that exposure might occur.

Stone marten (*Martes foina*) in Germany

In August 2001, a stone marten was found alive in a garden in Sachsen-Anhalt, Germany. It showed no clinical signs except a lack of timidity and reluctance to move. After several attempts to scare the animal away, it tried to attack a resident and was subsequently killed. Although the animal was negative by the fluorescent antibody test, it was later found to be positive by PCR for the presence of an EBLV-1 [9].

This case is the only record of an EBLV-1 infection detected in a wild animal other than a bat. It is speculated that this spillover infection may have arisen from the animal attacking an infected grounded bat and receiving a bite from the bat. Stone martens are known sometimes to prey on bats at winter hibernation sites.

EBLV-2

The first isolate of EBLV-2 in 1985 was from a Swiss bat biologist who had been working on bats in Finland [27]. Then, in 1986, it was isolated in Denmark and Germany from Daubenton's bats (*Myotis daubentonii*) and in Denmark from a pond bat (*Myotis dasycneme*). In total there have been 17 records of this virus, from Denmark, Finland, Germany, The Netherlands, Switzerland, United Kingdom and Ukraine. The principal, if not sole, natural wild hosts of EBLV-2 are *M. dasycneme* [48] and *M. daubentonii* [49]. These two species are closely related and, together with the *Myotis capaccinii* of Southern Europe, comprise the European members of the subgenus *Leuconoe*. The large and widespread genus of *Myotis* does not readily separate into subgenera, but *Leuconoe* is one of the more readily distinguishable groups. Both species are closely associated with open water as foraging habitat. In addition, two viruses from Central Asia have been described and may be close to, or variants of, EBLV-2 [25]. Epidemiological studies have also shown that there are at least four separate foci of EBLV-2. These include EBLV-2a in *M. dasycneme* in The Netherlands and *M. daubentonii* in the United Kingdom, a third focus from a human case of EBLV-2b in Finland and a fourth of EBLV-2b in *M. daubentonii* in Switzerland. As there have only been two cases of EBLV-2b, it is not possible to associate the virus with either species of *Myotis*

[19]. EBLV-2 was recognized as a separate genotype by Bourhy et al. [14] and was subsequently separated into two variants, EBLV-2a and EBLV-2b [19, 34]. Initially, it appeared that these variants were separated by host species (EBLV-2a in *M. dasycneme*; EBLV-2b in *M. daubentonii*). If the separation into two variants is valid, it no longer seems to be related to the host species; neither is it related to distribution.

Spillover infections of EBLVs in man

In Europe, only four human deaths from EBLVs have been reported up to 2002. There was no record of prophylactic immunization against rabies in any of these cases.

Voroshilovgrad (currently Lugansk), Ukraine

The first case occurred after a 15-year-old girl was bitten on the finger during the daytime by a bat of unknown species in Voroshilovgrad, Ukraine, in 1977 [1]. A lyssavirus was isolated from the girl's brain, and was thought to be an EBLV-1; however, the virus was never typed genetically.

Russia

The second death occurred in Russia in 1985 [4] after an 11-year-old girl 'Yuli' was attacked and bitten on the lower lip by a bat, which then flew away. Post-exposure treatment (PET) was not administered and the girl died 4–5 weeks after the reported date of exposure having shown symptoms typical of rabies. An EBLV-1 was isolated from brain autopsy samples [14].

Helsinki, Finland

The third case was a 30-year-old Swiss biologist (mentioned earlier) who was admitted to Helsinki University Central Hospital, Finland in 1985 with ascending paralysis and pain in the right arm and neck. Bats of different species in Malaysia had bitten him 4 years previously, and also in Switzerland 1 year before his death. He died of a rabies-like illness 20 days after hospital admission. He had never been vaccinated against rabies and received no PET before falling ill. An EBLV-2b virus was isolated from the brain. This was the first isolation of EBLV-2 and the first confirmed case of an EBLV-2 infection in a human following exposure to bats [2, 3].

Angus, Scotland

A 55-year-old bat conservationist was admitted to a hospital in Dundee, Scotland in November 2002, with acute haematemesis. He gave a 5-day history of pain and paraesthesia in the left arm followed by increasing weakness of his limbs with evidence of an evolving encephalitis with cerebellar involvement. The patient had never been vaccinated against rabies and did not receive PET. Saliva samples proved positive and a strain of EBLV-2a was identified. This fatal incident is only the second confirmed case of an EBLV-2 infection in a human following exposure to bats [5].

CLINICAL SIGNS

EBLV infection in humans

In the two human cases [3, 5] with documented clinical observations, both were suggestive of the 'furious' form of rabies common in patients infected with classical RABV. During the early stages of the infection, clinical symptoms include persistent limbic pain and pruritus at the bite site and paraesthesiae. A developing weakness in both upper limbs leads to increasing flaccid weakness of all limbs with hyporeflexia. Pain from the neck is usually reported from the onset of symptoms, which increases in intensity before becoming permanent. A pneumothorax requiring ventilatory support has been observed. Clinical symptoms progress rapidly and neurological impairment increases as the virus spreads through the central nervous system. Other symptoms include respiratory spasms and breathing difficulties, hyperexcitability, anxiety, convulsions, profuse salivation, altered perception, acute confusion and aggression leading to coma.

EBLV infection in bats

Clinical signs of weight loss, lack of coordination, muscular spasms, agitation and aggression have been reported from EBLV-positive bats [41, 42]. Hypersalivation was not reported from the two EBLV-2 bat cases in the United Kingdom. The distribution of RABV strains in bats demonstrates that the virus is disseminated to all tissue/organs tested with the exception of wing tissue (Table 3). The distribution of EBLVs from the brain to all peripheral organs including the salivary glands is identical to that in RABV-infected mammals.

Table 3. *Lyssavirus distribution in bat tissues*

	Study							
Tissue	EBLV-2	EBLV-1	ABLV	RABV	EBLV-1	EBLV-1	EBLV-1	EBLV-1
Brain	+	+	+	+	+	+	+	+
Salivary gland	n.d.	n.d.	+	+	n.d.	+	+	+
Tongue	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pharynx/larynx	n.d.	+	n.d.	n.d.	+	n.d.	n.d.	n.d.
Lung	n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d.	n.d.
Stomach	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Intestine/rectum	+	n.d.	+	n.d.	n.d.	+	n.d.	n.d.
Kidney/bladder	+	−	n.d.	n.d.	n.d.	+	n.d.	n.d.
Liver	+	−	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Heart	+	−	n.d.	+	n.d.	n.d.	n.d.	n.d.
Testis/ovary	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	n.d.
Brown fat*	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	+
Spleen	n.d.	−	n.d.	n.d.	n.d.	n.d.	n.d.	−
Fetus	n.d.	n.d.	n.d.	n.d.	n.d.	−	n.d.	n.d.
Wing/skin	−	n.d.	n.d.	n.d.	n.d.	n.d.	−	n.d.
Reference	[41]	[20]	[64]	[65]	[66]	[47]	[44]	[67]

* Hibernation link/chronic infection.

TRANSMISSION

It is not clear how EBLVs are transmitted between bats within a colony. The complex social behaviour of bats including mutual grooming and biting [50] may possibly maintain virus dissemination through the sharing of saliva. It is, however, speculated that the mechanisms of EBLV transmission via the oral route and the viral load required may result in a 'silent' infection. The possibility exists that bats might act as 'asymptomatic viral carriers' resulting in a non-clinical infection. Virus re-activation may occur as a result of specific factors including pregnancy and stress that cause immunosuppresion. As part of the intricate behaviour of bats, biting incidents are also fairly common, most possibly resulting in conventional viral transmission and infection.

Cryptic transmission of EBLVs

In the Americas, cryptic transmission of RABV bat variants is common and the bite is unrecognized [51–54]. It is conceivable that in Europe, some viral encephalitides of unrecognized aetiology might be due to EBLV as a result of a bite from a bat.

Direct (salivary) transmission of EBLVs

The presence of virus in the salivary glands, tongue and pharynx of the bat appears to be important because the most common forms of virus spread are a bite, lick (on broken skin) or contact with mucous membranes. All four reported human cases of EBLV documented previous exposure to bat bites. Two of the four cases caused by an EBLV reported a specific biting incident from a single bat. Both human cases of rabies caused by an EBLV-2 reported multiple exposure to bats that involved multiple biting incidents. Interestingly, a large number of bat handlers working throughout Europe have reported biting incidents from EBLV-infected bats without subsequent infection. It is possible that EBLV infection may occur infrequently due to the low level of saliva transmission. This suggests that bat-to-human spread of EBLVs may require a significantly higher viral load before an active infection is established compared to the RABV load received from an infected dog bite. The pathogenesis of EBLVs appears to be different, EBLV-2 being less virulent than EBLV-1 in a new host.

Horizontal transmission of EBLVs

Although frequent urination and defaecation is commonplace amongst bats, and a large amount of guano may be present within caves, resulting in the possibility of aerosolized virus, it is still doubtful whether EBLV spread among bats in caves is as a result of inhaled virus. Such aerosol transmission is most likely to be saliva in the air rather than excreta.

It is clear, however, that the latter route cannot be ignored. Aerosol transmission may be more likely in such caves with large numbers of bats, although even in caves in the United States there is little evidence for the occurrence of aerosol transmission of bat variants of RABV [51–53]. In natural conditions, transmission of EBLVs amongst bats sharing the same colony is more likely through mutual grooming (allogrooming), in which bites are incurred, in contrast to inhalation of infectious virus particles. In addition, during the mating season (mid to late autumn), 'aggressive mating' is well recorded in some species, where the male bat bites the female on the nape of the neck, which can serve as a means of transmitting infectious saliva to susceptible hosts. Aggressive behaviour for roost defence may also lead to biting incidents between bats.

Transmission studies of EBLV-1 in bats

Because of the protected status of bats in Europe, only one bat-to-bat transmission study has been reported, from Russia. In 1992 two EBLV-1 viruses (known as Yuli and Stade) and 'an unusual' RABV from a *Vespertilio murinus* bat were inoculated intramuscularly into a mixed group of 100 *M. daubentonii* and 11 *M. brandtii* bats and into 15–20 g adult white mice. Results showed that bats were less susceptible than mice to the genotype 5 viruses and that incubation periods were longer. Conversely, the susceptibility of bats inoculated with the 'unusual' genotype 1 virus was higher than that of mice, although the incubation period was significantly longer. It was also noted that whereas 50% of the bats inoculated with the genotype 5 viruses showed aggressive behaviour and convulsions, all bats inoculated with the 'unusual' genotype 1 virus showed only the paralytic form of lyssavirus infection.

Kuzmin & Botvinkin [55] inoculated *Pipistrellus pipistrellus* bats with Aravan virus, the above-mentioned *Vespertilio* and virus from the Yuli case. About 46 bats were treated with each virus. Between 17 and 47% of the bats became infected and died; incubation periods ranged from 13 to 67 days and duration of clinical signs from 1 to 13 days.

PREVENTION

Public health measures

Those with occupational exposure to bats including bat rescuers/rehabilitators are a high-risk group for exposure to EBLV. Prophylactic vaccination is recommended for those professionally or recreationally exposed to bats in most European countries. In the United Kingdom, it has become compulsory for licensed bat workers to be vaccinated against rabies.

Cross-protection of current vaccines against EBLV infection

Commercial vaccines for rabies (human and veterinary) are based on and protect against RABV. No person administered pre-exposure immunization or timely PET has died of rabies regardless of the source and genotype of the virus. This suggests that the RABV vaccines induce antibodies that should be capable of cross-neutralizing and cross-protecting against at least some lyssavirus genotypes.

The level of protection as determined in mice that survived after 28 days following a heterologous virus challenge using a non-genotype 1 lyssavirus appears to depend on the virus strain used in the vaccine, e.g. Pasteur virus (PV) or Pitman–Moore (PM) strain and the genotype of the challenge virus used [27, 56–61]. Generally, rabies vaccines based on the PM strain induced weaker protection against EBLV-1 than the PV strain, and few data are reported for EBLV-2 [57, 58]. To date there has not been a comprehensive study of the efficacy of currently available vaccines for EBLV infection. Most previous studies have been fragmentary and have examined the cross-protection issue using the most severe, and unnatural, intra-cranial challenge route.

In an early study [57], mice immunized intraperitoneally with the inactivated adjuvanted veterinary vaccines (Rabisin and Rabiffa) were protected against EBLV-1 challenge. However, Rabisin, Rabiffa and HDCV elicited only partial protection against EBLV-2.

Protection experiments were performed in mice using different inactivated vaccines [58]; the fixed RABV strains: PM, PV and LEP (Flury LEP) against an intracerebral challenge with EBLV-1. All three vaccines protected mice against challenge with CVS (Challenge Virus Standard). Vaccines prepared with PV, protected mice against EBLV-1 challenge. In contrast, PM or LEP vaccines did not protect mice against EBLV-1 infection.

In an immunogenicity study [59], T- and B-cell human responses to EBLV-1 induced by post-exposure rabies vaccination (PM virus vaccine) were evaluated by measuring EBLV-1-specific neutralizing

antibody titres. On day 21, the vaccine induced CVS-specific neutralizing antibodies in all patients; but EBLV-1-specific neutralizing antibodies were induced in only 73% of patients. Patients having EBLV-1-specific neutralizing antibodies were usually those in whom vaccination induced high titres of CVS-specific neutralizing antibodies. Rabies vaccination induced neither T- nor B-cell EBLV-1-specific responses in 22% of patients.

In vivo testing in mice for efficacy against intracerebral challenge with the Dutch bat EBLV-1 resulted in acceptable levels of protection with 4 out of 5 veterinary vaccines currently available in The Netherlands [60].

TREATMENT

Rabies vaccine and anti-rabies immunoglobulin are recommended for humans bitten by an EBLV-positive bat. In the United States, the advice is that any bat involved in a bite or scratch incident is retained for diagnostic testing.

CONCLUSION

In light of the prevalence of EBLVs in bats throughout Europe, the risk of a member of the general public with little direct contact with bats contracting a lyssavirus infection from a European bat is low; however the risk should not be underestimated. The risk to individuals who routinely handle and are bitten by insectivorous bats in Europe is significantly higher. All bat handlers should be encouraged to wear bite-proof gloves and to complete a course of rabies immunizations before handling bats. In the United Kingdom, all bat handlers are offered free rabies pre-immunizations and booster injections. The recognized prevalence of EBLVs in Europe underlines the need for all individuals who are bitten by a bat in the course of either their occupation or recreation to wash the bite site and seek immediate vaccination.

It is plausible that the pathogenesis of EBLVs is different, with EBLV-2 being less virulent than EBLV-1 in a new host. Apart from the four human EBLV cases described, 'spillover' infections of EBLVs to terrestrial mammals occur, though uncommonly. They have never been reported for EBLV-2 and only three cases in mammals have been reported for EBLV-1.

Research is still required to further understand the role that insectivorous bats play in the virus–host relationship and subsequent transmission of EBLVs. Previous reports have suggested that insectivous bats may harbour an EBLV for extended periods of time whilst the bat shows no obvious clinical signs ('silent infection') [45]. Although it is feasible that EBLVs remain in the host in a 'latent' (dormant) state for long periods of time with potential 'asymptomatic carriage', it is not clear why the virus–host relationship of EBLVs would differ from classical RABV infection in other mammals, including insectivorous bats [62]. It is plausible that a particular stimulant causes the virus to be activated following a period of virus suppression. The trigger for virus reactivation is not known although it can be speculated that immunosuppression, possibly as a result of pregnancy, may enhance virus replication [63]. Interestingly, the first case of EBLV-2 in the United Kingdom [42] was identified in a pregnant Daubenton's bat. Additionally, the second case in the United Kingdom [41] was a juvenile bat, estimated to be only a few weeks old. Both cases indicate a causal association with pregnancy and likely immunosuppression. The exact features that the virus undergoes in the host are not fully understood and whether it remains dormant in immune-privileged sites, i.e. neuronal tissue or brown fat is doubtful.

In the United Kingdom, a study of 'passive surveillance' for lyssaviruses in bats has been under way since 1987. Both EBLV-positive bats identified in the United Kingdom, however, were reported as 'suspect' bat cases and were not identified through 'passive surveillance', which can be biased and may lack sensitivity.

In order to fully assess the true prevalence of EBLVs in British bat populations and a better understanding of the public health risk that EBLV- infected bats pose, a targeted 'active' surveillance programme has been initiated to complement the 'passive surveillance' of British bats. The primary aim of this study will be to assess the exposure of target bat species (namely *Myotis daubentonii* and *Eptesicus serotinus*) to EBLVs throughout the United Kingdom.

These studies will be performed in close collaboration with bat conservationists in order to support public health issues whilst protecting the welfare of bats and further contributing to our understanding of the true prevalence of EBLVs throughout Europe.

ACKNOWLEDGEMENTS

The Department for the Environment, Food, Rural Affairs (Defra), UK is the principal sponsor for all rabies work at the Veterinary Laboratories Agency.

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