

Workpackage 5: Final Research Report

WP number	5
Title	Molecular epidemiology of European bat lyssaviruses
WP Leader	Anthony FOOKS
Name and Address	Rabies and Wildlife Zoonoses Group Veterinary Laboratories Agency (VLA) Woodham Lane, New Haw Surrey, KT15 3NB UNITED KINGDOM
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Summary

The first international network of scientists involved in European bat lyssavirus (EBLV) research and surveillance has been established. Two meetings in September 2004 (Amsterdam) and 2005 (Krakow) highlighted differences in the way institutes approached passive and active bat surveillance, both in terms of field work and diagnostic methods. As a result, draft guidelines have been produced in an attempt to standardize surveillance systems. They have been circulated amongst participants and await publication in *Rabies Bulletin Europe*. In addition, the joint EU-OIE-WHO 1st 'Rabies in Europe' meeting (Kiev 2005) incorporated the recommendations of this Workpackage in their final report (www.oie.int).

Genetic sequencing of bat lyssaviruses is currently performed in several partner institutes of Med-Vet-Net. Data previously held by individual institutes, or accessible only through extensive searches of multiple manuscripts and sequence databases, is now available via a single source. The first integrated EBLV database, incorporating the clinical details, laboratory investigations and virus sequences of EBLV cases in Europe has been developed under Workpackage 5 and placed on the private Med-Vet-Net website. This database is accessible to external experts on request. The surveillance guidelines and EBLV database will be invaluable to researchers and diagnosticians currently involved in, or those wishing to initiate, lyssavirus surveillance in bats in their respective countries.

Bat variants of rabies virus: European bat lyssavirus

European bat lyssaviruses (EBLVs) are RNA viruses and are members of the *Lyssavirus* genus of the family *Rhabdoviridae*. EBLVs are most adapted to European species of insectivorous bat and are genetically different to the 'classical rabies virus' strains, historically isolated from dogs, cats and foxes.

European bat lyssavirus type-1 (EBLV-1) has been isolated principally from Serotine (*Eptesicus serotinus*) bats and EBLV type-2 from Myotis species (*Myotis daubentonii* and *M. dascyneme*) bats throughout Europe. Currently, there have been >700 reported cases of EBLV in European bats (Figure 5.2); 95% of these have been EBLV-2 isolates from Serotine bats and the remainder have been EBLV-2. On rare occasions, these strains have been known to spillover between bats and other animals. On two occasions sheep have been infected with EBLV-1 in Denmark and the same strain was detected in a stone marten in Germany. The risk of EBLV exposure to humans is low, especially in individuals who do not handle bats, however, since 1977 there have been five (3 confirmed; 2 suspect) human deaths from rabies in Europe attributed to EBLV infections. The latest human case was a bat conservationist from Scotland who was infected with EBLV-2 and later died of rabies. These cases all occurred in humans had been exposed to bats, usually through a biting

immunized against rabies and even if they are not, post-exposure vaccination is known to be effective, if administered soon after exposure. Daubenton's bats rarely roost in houses and so are unlikely to cause widespread problems in man. The public are advised to avoid handling bats and if bitten or scratched by a bat should immediately wash the affected area with soap and water and seek medical attention.

Where EBLVs are found

There are two types of EBLV (Figure 5.1). Type 1 (genotype 5) which has subtypes a and b of which Type 1a is the most common and has been found across northern and central Europe to Russia. Type 1b has been isolated in some Western countries down to Spain. EBLV type-2 (genotype 6) also has subtypes a and b but has rarely been identified, with one case of EBLV-2a found in The Netherlands and some cases found in the UK and Switzerland. Both types of EBLV cause similar rabies-like encephalitis (inflammation of the brain) in man, however it is possible that differences in their glycoprotein content may influence the origin and development of the disease.

How the virus replicates

The incubation period of lyssavirus is typically 20–90 days, although periods ranging from a few days to more than a year have been documented. The virus replicates in local muscle fibres and binds to receptors in the neuromuscular junction. It

then travels rapidly to the central nervous system, replicates in the neurons of the spinal cord and dorsal root ganglia, infects brain neurons and then spreads along nerves to the major exit portals, the salivary glands. The cause of rabies encephalitis was believed to be unique until 1956 when the first rabies-related viruses were isolated in Africa and Europe. Continuing developments in molecular biology allowed the identification of the lyssavirus genus and the classification of seven genotypes, six of which have caused rabies encephalomyelitis (inflammation of the brain and spinal chord) in humans or animal deaths in nature.

Re-emergence of the virus

It is possible that healthy bats may be infected and infectious before clinical signs appear. Some bats in Europe have been shown to recover from exposure, become seropositive and survive suggesting that an 'atypical infection' has occurred. Viral RNA has been detected in the brain of Spanish bats without evidence of viral replication. This suggests that re-emergence of the virus is possible.

Objectives

The overall objective of Workpackage 5 was to provide accurate information on the risk of bat-associated rabies in Europe to animal and human health by forging collaborations among rabies laboratories. Initially, the goal was to capture all of the genetic information from the various collaborating institutes across Europe and to store this information on a common database for use by all members of Med-Vet-Net and access by others outside of the network. Rabies is a notifiable disease throughout Europe and it is possible that under-reporting of bat rabies cases is common. The principal aim was to assess the risks that EBLVs pose and the risk of spillover to domestic livestock, which ultimately affects the food chain. This occurrence of events is common in Latin American

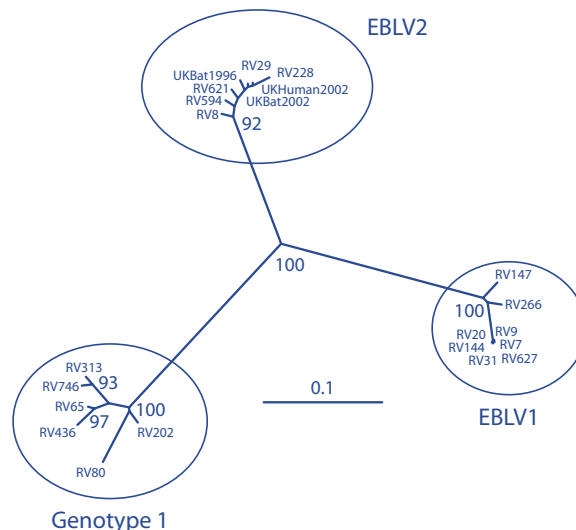


Figure 5.1 Radial tree phylogenetic analysis of viral isolates from genotypes 1, 5 and 6 using a 400 bp sequence of the lyssavirus nucleoprotein coding sequence.

Objectives

The overall objective is to provide accurate information on the risk of bat-associated rabies in Europe to human health by forging collaborations among rabies laboratories. This will be achieved by:

- Collation of sequence data and archived material from EBLV isolates detected by Med-Vet-Net partner institutes. As far as possible viruses will also be obtained from associate institutes throughout Europe and neighbouring countries.
- Setting up a database of sequence data for the EBLV isolates. Primarily the database will include partial N gene sequence data. However, this could be enhanced with sequence data from other genomic regions
- Disseminating the information electronically via the Internet. This will facilitate the interpretation and publication of sequence data to enable rapid genotyping of new viruses and provide a greater understanding of the geographical and host-specific evolution of the European bat lyssaviruses.

countries where a genotype 1 bat variant of classical rabies virus will commonly spillover from the vampire bat host (*Desmodus rotundus*) to cattle, horses and on rare occasions to man. This causes both economic losses and a risk to public health. Our working hypothesis is to question whether EBLVs are less virulent than other bat variants of rabies virus and whether the risk of spillover is therefore smaller. Evidence from North America has shown that genotype 1 bat variants that have spilled over from bats to terrestrial mammals have caused minor epizootics of rabies. With the reduction of vaccination of foxes against rabies throughout Europe and the subsequent increase in fox numbers, the possibility exists that a rabies epizootic in wildlife could occur.

The objectives of Workpackage 5 were to be achieved by:

1. Collection of sequence data and archived material from EBLV isolates detected by Med-Vet-Net Partner Institutes.

Table 5.1 EBLV isolates submitted to participating laboratories

Group	EBLV-1	EBLV-2
VLA – UK	46 (all sequenced)	12 (all sequenced – 4 UK)
AFSSA – France	18 (all sequenced)	1 (from VLA)
ISCII – Spain	20+ (part sequenced)	0
SVA – Sweden	0 (no sequencing)	0 (no sequencing)
FRC – Germany	160 (35–40 sequenced N-P)	2
DFVF – Denmark	200 (none sequenced)	0
PZH – Poland	13+ (3 sequenced)	0
RIVM – Netherlands	50+ (50 sequenced)	6 (5 sequenced)

2. Setting up a database of sequence data for the EBLV isolates.

3. Dissemination of the necessary information electronically via the Internet.

Agreeing optimal approaches

A meeting was held in Amsterdam in September 2004. Representatives from all participating institutes were present.

Guidelines for active and passive bat surveillance were agreed. Summaries of the bat, virus and sequence archives held at the relevant institutes were presented and the optimum database design features were agreed.

A second meeting was held in Krakow in September 2005 to discuss progress and evaluate the prototype database.

Problems with EBLV reverse transcription (RT)-PCR and sequencing methods were discussed. Currently RT-PCR is used to identify and sequence isolates and the importance of using primers, which will pick up all strains of EBLV was highlighted. Currently the 'N' gene is targeted but it was suggested that this gene is too conserved so isolates within genotypes are not necessarily distinguished. Some suggestions to target the 'G' gene were discussed. In the meantime it was agreed that a 400 base pair region of the N gene would be used by everyone and use of the G gene would be considered at a later date.

Creating and utilizing the EBLV Database

The availability of isolate data was assessed by questionnaire. The number of isolates held by each partner institute at that time is reported in Table 5.1.

In conjunction with the Communications Unit, a prototype database was designed with secure access via the Med-Vet-Net website. An online submission form for data entry was produced. The optimum methods to ensure security and facilitate authorization of submitted data online was determined. All participants likely to submit data were asked to test the submission form and database by submitting a dummy case. Constructive feedback was obtained from a small number of participants resulting in some minor adjustments to the design of the submission form. Data submission was initiated in March 2005.

Major achievements

1. This Workpackage has facilitated the establishment of the first network of European scientists involved in bat *lyssavirus* surveillance.
2. A unique database has been designed incorporating the clinical details, laboratory investigations and virus sequences of EBLV cases in Europe. This database will be accessible to interested scientists on request.
3. Common approaches to both active and passive bat rabies surveillance programmes in Europe have been determined and have been published in *Rabies Bulletin Europe* (WHO).
4. Opportunities for training and collaboration with respect to both research and surveillance programmes have been exploited by Med-Vet-Net participants.

The prototype database has been sited on a private page of the Med-Vet-Net website. With the approval of all participants the database will be made available to interested scientists via the website with restricted access, e.g. they will not have access to unpublished sequence data.

Benefits of the European network

Collaborations, both for research and surveillance, have been initiated as a direct result of establishing this network of scientists. One unanticipated benefit of this network was the generation of draft bat surveillance guidelines. These guidelines have been published (*Rabies Bulletin Europe* (WHO) http://www.who-rabies-bulletin.org/q4_2005/contents/3.1.html) and will be of great benefit to the European community involved in *lyssavirus* surveillance. In particular the network has considered the following relevant issues:

1. The responses to a questionnaire on EBLV surveillance highlighted that the species of bat sampled is often not recorded. It is considered particularly important to indicate the specific bat species when publicising rabies data, rather than implicating all bats.
2. National data about bat species should be centralized in a WHO designated laboratory for Europe and consistency of this data both at the European and national levels was a problem. For

example in Germany the 16 states have different submission policies.

3. A harmonized approach for passive surveillance and bat identification should be established.

4. It was recommended that more co-operation with bat conservation organizations throughout Europe is required. In particular, European rabies scientists should work with the EUROBATS consortium. EUROBATS is a European group of bat workers, conservationists, wildlife officers and ecologists, who work together to ensure the continued protection of bats across Europe. They also recommend ways of working with bats, furthering our understanding of bat species and their behaviour. In addition, European rabies scientists should liaise with national bat groups when planning and implementing surveillance programmes. This collaboration has been published in the *Rabies Bulletin Europe* (RBE).

5. Guidelines for Active and Passive surveillance should be drafted and circulated to all participants for comment prior to their publication in the RBE.

6 The status of rabies as a notifiable disease should be re-emphasized and publicized within each country – this point was first proposed by the WHO Expert Committee on Rabies at a meeting held in Geneva in October 2004.

7. Obtaining data from non-bite/scratch cases was noted as a problem in all countries and active surveillance was recognized as the one way to stop this bias.

8. At present, there is no information on the impact of social behaviour on the transmission of EBLV or the mating behaviour of the European long-eared bat. Therefore, future information about different environments/habitats and how they effect EBLV transmission is needed.

In conclusion, this Workpackage has facilitated the establishment of the first network of European scientists involved in bat *lyssavirus* surveillance and provided a unique database of virus sequences of EBLV cases in Europe. An additional output has been the development of common approaches to both active and passive bat rabies surveillance programmes in Europe.

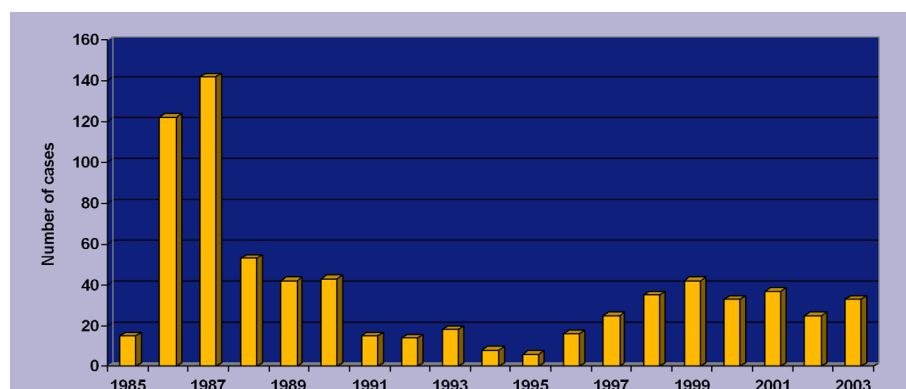


Figure 5.2 Rabies cases in bats, Europe (1985–2003).