

# Novel Lyssavirus in Bat, Spain

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A new tentative lyssavirus, Lleida bat lyssavirus, was found in a bent-winged bat (*Miniopterus schreibersii*) in Spain. It does not belong to phylogroups I or II, and it seems to be more closely related to the West Causasian bat virus, and especially to the Ikoma lyssavirus.

Bats have been considered natural hosts of a wide diversity of viruses, including human pathogens such as lyssaviruses, severe acute respiratory syndrome coronavirus, henipavirus, and filoviruses (1). Within the genus *Lyssavirus*, 12 species have been described: *Rabies virus* (RABV), *Lagos bat virus* (LBV), *Mokola virus* (MOKV), *Duvenhage virus* (DUVV), *European bat lyssavirus* types 1 and 2 (EBLV-1 and -2), *Australian bat lyssavirus* (ABLV), *Aravan virus* (ARAV), *Khujand virus* (KHUV), *Irkut virus* (IRKV), *West Causasian bat virus* (WCBV), and *Shimoni bat virus* (SHIBV). Two more recently described viruses have not yet been classified: Bokeloh bat lyssavirus (BBLV) (2) and Ikoma lyssavirus (IKOV) (3).

Bats are the natural reservoirs for most lyssaviruses, and to our knowledge, only MOKV and IKOV have never been detected in bats. RABV is the only virus known to establish epidemiologic cycles in bats and carnivores, and it is responsible for most human infections, mainly transmitted by dogs. The genus *Lyssavirus* comprises at least 2 phylogroups: phylogroup I (RABV, DUVV, EBLV1–2, ABLV, ARAV, IRKV, BBLV, KHUV) and phylogroup II (LBV, MOKV, and SHIBV). Phylogroup III consists of WCBV (4). According to a recent phylogenetic reconstruction that included the novel IKOV and was based on a fragment of 405 nt from the nucleoprotein gene, IKOV has

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proven to be highly divergent (3) and probably also forms part of phylogroup III.

During 1977–2011 in Europe, 988 cases of bat rabies were reported to the Rabies Bulletin Europe. Bats of the species *Eptesicus serotinus* and *E. isabellinus*, which account for >95% of cases, are considered the major natural reservoirs of EBLV-1. Several bat species within the genus *Myotis* are reservoirs for EBLV-2, BBLV, and the central Asian lyssaviruses ARAV and KHUV (5). WCBV has been isolated in the common bent-winged bat *Miniopterus schreibersii* (6). Other bat species might act as eventual hosts, although in Spain, bat rabies has been declared only in *E. isabellinus* bats (7). The possibility of a wider host range has been suggested by some surveys on natural bat colonies of other bat species describing neutralizing antibodies and genomic fragments related to EBLV-1 (8).

## The Study

In July 2011, a bat was found in the City of Lleida and taken to the Wildlife Care Center of Vallcalent (Lleida, Catalonia). The bat arrived lethargic and dehydrated, died soon after admission, and its carcass was frozen at -20°C. On March 12, 2012, as part of the rabies surveillance program in Spain, the bat carcass was received by the National Center of Microbiology, where rabies testing was conducted by 2 generic reverse transcription PCR (RT-PCR) methods for lyssavirus detection (9,10) and 2 commercial rabies antiserum assays (Bio-Rad Laboratories, Marnes La Coquette, France; and Fujirebio, Inc., Tokyo, Japan) for antigen detection by fluorescent antibody testing.

Brain smears were positive for lyssavirus by RT-PCR and fluorescent antibody testing, and an oropharyngeal swab sample was positive by RT-PCR. Further attempts to isolate the virus by tissue cultures were unsuccessful after 2 blind passages in BHK-21 and murine neuroblastoma cells. The negative results could be explained by the fact that the sample had been stored at -20°C for 8 months and had been frozen and thawed twice before cell culture testing; however, the possibility of the cell lines not being permissive for the virus cannot be excluded.

The bat was morphologically identified as a bent-winged bat (*M. schreibersii*) and genetically identified by cytochrome b sequencing (11). The genomic sequence of the corresponding fragment of the diagnostic RT-PCR on the conserved region of the nucleoprotein gene, determined by BLAST (<http://blast.ncbi.nlm.nih.gov/>), showed no substantial sequence similarity to previously known lyssaviruses.

To determine the identity of the lyssavirus, we sequenced a larger fragment (565 bp), including the variable coding region of the nucleoprotein gene (GenBank accession number submitted). We reconstructed an overall



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