

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 codifying region of the nucleoprotein gene (GenBank accession number submitted). We reconstructed an overall

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References

- Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. *Clin Microbiol Rev*. 2006;19:531–45. <http://dx.doi.org/10.1128/CMR.00017-06>
- Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, Keller B, et al. Novel lyssavirus in natterer's bat, Germany. *Emerg Infect Dis*. 2011;17:1519–22. [10.3201/eid1708.110201](http://dx.doi.org/10.3201/eid1708.110201).
- Marston DA, Horton DL, Ngeleja C, Hampson K, McElhinney LM, Banyard AC, et al. Ikoma lyssavirus, highly divergent novel lyssavirus in African civet. *Emerg Infect Dis*. 2012;18:664–7. <http://dx.doi.org/10.3201/eid1804.111553>
- Kuzmin IV, Wu X, Tordo N, Rupprecht CE. Complete genomes of Aravan, Khujand, Irkut and West Caucasian bat viruses, with special attention to the polymerase gene and non-coding regions. *Virus Res*. 2008;136:81–90. <http://dx.doi.org/10.1016/j.virusres.2008.04.021>
- Schatz J, Fooks AR, McElhinney L, Horton D, Echevarria J, Vázquez-Moron S, et al. Current state of bat rabies surveillance in Europe. *Zoonoses Public Health*. 2013;60:22–34. <http://dx.doi.org/10.1111/zph.12002>
- Botvinkin AD, Poleschuk EM, Kuzmin IV, Bosisova TI, Gazaryan SV, Yager P, et al. Novel lyssaviruses isolated from bats in Russia. *Emerg Infect Dis*. 2003;9:1623–5. <http://dx.doi.org/10.3201/eid0912.030374>
- Vázquez-Morón S, Juste J, Ibáñez C, Berciano JM, Echevarria JE. Phylogeny of European bat lyssavirus 1 in *Eptesicus isabellinus* bats in Spain. *Emerg Infect Dis*. 2011;17:520–3. <http://dx.doi.org/10.3201/eid1703.100894>
- Serra-Cobo J, Amengual B, Abellán C, Bourhy H. European bat lyssavirus infection in Spanish bat populations. *Emerg Infect Dis*. 2002;8:413–20. <http://dx.doi.org/10.3201/eid0804.010263>
- Echevarria JE, Avellón A, Juste J, Vera M, Ibáñez C. Screening of active lyssavirus infection in wild bat populations by viral RNA detection on oropharyngeal swabs. *J Clin Microbiol*. 2001;39:3678–83. <http://dx.doi.org/10.1128/JCM.39.10.3678-3683.2001>
- Vázquez-Morón S, Avellón A, Echevarria JE. RT-PCR for detection of all seven genotypes of *Lyssavirus* genus. *J Virol Methods*. 2006;135:281–7. <http://dx.doi.org/10.1016/j.jviromet.2006.03.008>
- Ibáñez C, García-Mudarra JL, Ruedi M, Stadelman B, Juste J. The Iberian contribution to cryptic diversity in European bats. *Acta Chiropt*. 2006;8:277–97. [http://dx.doi.org/10.3161/1733-5329\(2006\)8\[277:TICTCD\]2.0.CO;2](http://dx.doi.org/10.3161/1733-5329(2006)8[277:TICTCD]2.0.CO;2)
- Hofer SR, Van der Bussche R. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropt*. 2003;5:1–63. <http://dx.doi.org/10.3161/001.005.s101>
- Serra-Cobo J, Sanz V, Martínez-Rica JP. Migratory movements of *Miniopterus schreibersii* in the north-east of Spain. *Acta Theriol (Warsz)*. 1998;43:271–83.
- Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Markotter W, Beagley JC, et al. Possible emergence of West Caucasian bat virus in Africa. *Emerg Infect Dis*. 2008;14:1887–9. <http://dx.doi.org/10.3201/eid1412.080750>
- Both L, Banyard A, van Dolleweerd C, Horton D, Ma JKC, Fooks AR. Passive immunity in the prevention of rabies: a neglected tool for a neglected disease. *Lancet Infect Dis*. 2012;12:397–407. [http://dx.doi.org/10.1016/S1473-3099\(11\)70340-1](http://dx.doi.org/10.1016/S1473-3099(11)70340-1)

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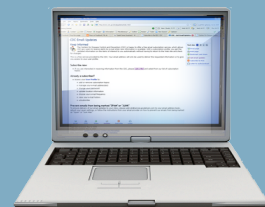
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Technical Appendix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. LLEBV		71.6	68.6	68.1	67.6	68.2	67.0	67.3	67.4	66.6	67.7	65.7	65.9	67.2	68.6	65.7	67.2	66.1	65.8	65.5	65.4	64.7	66.3	63.7	64.0
2. JN80050 IKOV	71.6		63.7	67.4	66.2	65.4	65.4	64.5	69.5	65.3	64.6	66.0	64.0	68.1	64.9	63.5	64.7	66.0	65.0	64.5	64.0	64.2	63.8	65.3	66.0
3. GU170201 SHIBV	68.6	63.7		73.6	73.1	72.3	71.4	75.8	71.1	69.6	71.1	75.8	76.0	76.8	80.0	73.3	74.3	72.8	71.9	71.6	71.1	71.4	72.3	70.9	69.6
4. EF614260 IRKV	68.1	67.4	73.6		73.8	75.8	75.1	72.3	68.6	71.6	74.3	71.1	70.9	72.9	72.6	70.4	72.7	73.6	77.8	77.8	73.6	73.3	70.1	76.8	77.0
5. EF614261 KHUV	68.1	66.7	73.1	73.8		79.8	77.8	77.3	67.5	72.8	79.0	70.1	68.6	72.1	67.9	67.9	67.2	77.3	73.1	72.6	75.6	73.1	72.6	72.3	72.3
6. NC 009528 EBLV2	68.4	65.9	72.3	75.8	79.8		95.6	76.8	70.7	72.8	75.3	71.4	68.9	72.8	70.1	68.4	70.2	78.3	72.8	73.1	72.1	70.6	70.6	72.6	71.6
7. AY863406 EBLV2	67.2	65.7	71.4	75.1	77.8	95.6		76.5	70.4	74.1	75.8	72.3	69.9	73.8	68.9	68.2	68.2	80.0	73.6	73.8	74.1	70.9	70.1	74.8	73.8
8. EF614259 ARAV	67.7	64.7	75.8	72.3	77.3	76.8	76.5		70.6	73.6	76.8	73.6	72.8	73.8	72.3	72.1	73.1	77.3	73.3	73.1	71.9	70.9	73.6	75.3	75.6
9. EF61425 WCBV	67.4	69.6	71.1	68.6	67.7	70.9	70.6	70.6		68.1	69.7	72.8	74.1	71.9	74.1	69.4	71.1	70.7	70.1	69.9	67.2	66.9	66.9	68.1	67.9
10. AF081020 ABLV	66.9	65.9	69.6	71.6	72.8	72.8	74.1	73.6	68.1		84.4	71.9	72.1	72.1	71.1	72.3	70.4	74.8	72.8	73.3	72.6	73.6	72.6	72.8	73.1
11. AF418014 ABLV	67.9	64.9	71.1	74.3	79.0	75.3	75.8	76.8	69.9	84.4		71.1	74.1	72.1	69.6	72.8	72.6	79.8	76.3	75.8	76.0	74.8	74.8	72.6	72.6
12. EF547447 LBV	65.7	66.2	75.8	71.1	70.1	71.4	72.3	73.6	72.8	71.9	71.1		78.0	79.3	79.8	76.8	74.1	71.1	69.6	69.9	74.1	71.4	73.3	71.4	72.3
13. EF547449 LBV	65.9	64.2	76.0	70.9	68.6	68.9	69.9	72.8	74.1	72.1	74.1	78.0		81.2	81.2	75.8	73.6	72.8	73.1	72.6	70.1	70.1	71.9	71.4	70.9
14. GU170202 LBV	67.2	68.6	76.8	73.1	72.1	72.8	73.8	73.8	71.9	72.1	72.1	79.3	81.2		78.8	72.6	71.9	70.4	72.8	72.1	71.4	72.3	73.1	71.4	72.3
15. EF547459 LBV	68.6	65.2	80.0	72.6	67.9	70.1	68.9	72.3	74.1	71.1	69.6	79.8	81.2	78.8		72.6	73.3	70.1	71.6	70.6	69.6	70.4	71.6	69.6	71.1
16. GU992313 MOKV	65.7	63.5	73.3	70.4	67.9	68.4	68.4	72.1	69.4	72.3	72.8	76.8	75.8	72.6	72.6		88.9	69.9	72.6	72.3	71.4	72.1	71.6	68.1	68.1
17. NC 006429 MOKV	67.2	64.7	74.3	72.8	67.2	70.4	68.4	73.1	71.1	70.4	72.6	74.1	73.6	71.9	73.3	88.9		69.6	72.4	71.9	70.2	70.5	69.9	68.5	68.7
18. JF311903 BBLV	66.4	66.7	72.8	73.6	77.3	78.3	80.0	77.3	70.9	74.8	79.8	71.1	72.8	70.4	70.1	69.9	69.6		72.6	72.3	75.1	72.3	73.1	74.1	73.6
19. EU293119 DUVV	65.9	65.2	71.9	77.8	73.1	72.8	73.6	73.3	70.1	72.8	76.3	69.6	73.1	72.8	71.6	72.6	72.6	72.6		99.0	72.6	70.9	70.6	78.8	78.0
20. EU293120 DUVV	65.7	64.7	71.6	77.8	72.6	73.1	73.8	73.1	69.9	73.3	75.8	69.9	72.6	72.1	70.6	72.3	72.1	72.3	99.0		73.1	70.9	71.1	78.0	77.3
21. FJ228497 RABV	65.4	64.0	71.1	73.6	75.6	72.1	74.1	71.9	67.2	72.6	76.0	74.1	70.1	71.4	69.6	71.4	70.4	75.1	72.6	73.1		92.3	86.2	72.1	71.6
22. NC 001542 RABV	64.7	64.2	71.4	73.3	73.1	70.6	70.9	70.9	66.9	73.6	74.8	71.4	70.1	72.3	70.4	72.1	70.9	72.3	70.9	70.9	92.3		85.4	70.9	69.6
23. GU991830 RABV	66.4	64.0	72.3	70.1	72.6	70.6	70.1	73.6	66.9	72.6	74.8	73.3	72.1	73.1	71.6	71.6	69.9	73.1	70.6	71.1	86.2	85.4		70.6	70.4
24. AY863392 EBLV1	63.7	65.4	70.9	76.8	72.3	72.6	74.8	75.3	68.1	72.8	72.6	71.4	71.4	71.4	69.6	68.1	68.6	74.1	78.8	78.0	72.1	70.9	70.6		95.1
25. EF157976 EBLV1	64.0	66.2	69.6	77.0	72.3	71.6	73.8	75.6	67.9	73.1	72.6	72.3	70.9	72.3	71.1	68.1	68.9	73.6	78.0	77.3	71.6	69.6	70.4	95.1	

Similarity
Identity