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Active sero-survey for European bat lyssavirus type-1 circulation in North African insectivorous bats

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Bats have specific biological and ecological characteristics that make them suitable hosts for viruses; some of these viruses have the ability to infect humans and other domestic and wild mammals. Among these zoonotic viruses, lyssaviruses are known to circulate among the European insectivorous bats. To date, 5 lyssaviruses species and one recently discovered species circulating in Europe have been recognized: European bat 1 lyssavirus (EBLV-1), European bat 2 lyssavirus (EBLV-2), Bokeloh Bat Lyssavirus (BBLV), Lleida bat lyssavirus (LLBV), West Caucasian Bat Lyssavirus (WVBV) and the tentative novel member of the genus Lyssavirus Kotalahti bat lyssavirus.

In Africa, to date, three lyssaviruses have been identified in bats: Lagos bat virus (LBV), reported for the first time in Nigeria (1956) from an Eidolon helvum; Duvenhage virus (DUVV), detected in South Africa (1970) from the Miniopterus genus; and the Shimon bat virus (SHIBV), isolated from Hipposideros vittatus in Kenya (2009). However, serological evidence of West Caucasian bat virus (WCBV) and EBLV-1 infection has been reported in bats from Kenya and southwestern Indian Ocean islands, respectively. Little is known about the circulation and distribution of insectivorous bat lyssaviruses in North Africa, as well as the impact such viruses may have on public health. Although there is no evidence that bats migrate between the two continents across the Mediterranean Sea, migratory species such as Miniopterus schreibersii can fly over the sea for long distances. The aim of this study was to assess the potential circulation of European bat lyssaviruses in Northern Africa from 2007 to 2012.

All bats were handled in strict accordance with good animal practices, as defined by the current European legislation. The capture of bats and blood-sampling were authorized with specific permits issued by the Forest Ministry from Morocco and Algeria.

Sampling sites were selected according to bat behavior and species, which were classified as synanthropic (urban areas), migratory and gregarious. Bat colonies were sampled throughout the year, although hibernation and birthing periods were deliberately avoided. Bats were randomly captured inside the roosts with long-handled butterfly nets in the daytime and with mist nets at sunset, when they emerged to forage, and only in cases where access to the roost interior was not possible. The individuals identified as juveniles were released without further sampling. Morphological identification of bats was performed in compliance with the identification keys for the bats of Europe, and the bats were sexed. Some Miniopterus were sampled in a specific location of the Great Atlas, where Miniopterus magrebensis lives. This bat species was described only after our fieldwork had been completed, which means that we had no way to retrospectively determine which individuals belonged to the...
species *M. maghrebensis*. For this reason, the *Miniopterus* bats sampled in the Atlas region were included in the *Miniopterus* complex reported in Table 1 (*M. maghrebensis*/*M. schreibersii*).

Blood samples (30–250 μL, depending on the bat’s size, and always less than 1% of the body mass) were obtained by a small puncture made next to the proximal epiphysis of the radius. The volume of blood was extracted in compliance with FAO guidelines. Vials containing blood were stored at 4 °C for a few hours. Samples were centrifuged for 20 min at 12,000 rpm, and sera were extracted with a micropipette. Serum samples and clot pellets were frozen at −20 °C until analysis. The technique used to detect lyssavirus neutralizing antibodies was an adaptation of the rapid fluorescent focus inhibition test (RFFIT). Titers were expressed as the arithmetic means of two independent repetitions. Samples were considered positive when the number of fluorescent foci was reduced by 50% at the 1:81 dilution, as previously described11. Bats that were found dead were collected in the field work.

### Table 1  Serological results of EBLV-1 analyses from North Africa (2007 to 2012) and comparison with serological results obtained from samples collected in South-West Europe (Spain and Italy) from 2007 to 2015

| Species                      | North Africa | South West Europe |
|------------------------------|--------------|-------------------|
|                              | Algeriaa     | Moroccoa          |
|                              | SIRb         | Spainb            |
|                              |              | Italyb            |
|                              |              | SIRb              |
|                              | Total        |                   |
| *F. Rhinolophidae*           |              |                   |
| Rhinolophus ferrumequinum    | 1/47         | 38/165            |
| Rhinolophus euryale         | 3/31         | 1/2               |
| Rhinolophus mehelyi         | nd           | nd                |
| Rhinolophus blasii          | 0/1          | nd                |
| Rhinolophus hipposideros    | nd           | nd                |
| *F. Vespertilionidae*        |              |                   |
| Myotis myotis               | nd           | nd                |
| Myotis blythii              | nd           | 18/105            |
| Myotis punicus              | 1/63         | 17/99             |
| Myotis capaccinii           | 0/6          | 0/2               |
| Myotis escalerae            | nd           | 6/18              |
| Myotis emarginatus          | 0/27         | 0/2               |
| Myotis daubentoni           | nd           | nd                |
| Myotis mystacinus           | nd           | 0/1               |
| Pipistrellus pipistrellus   | nd           | 7/39              |
| Pipistrellus kuhlii         | nd           | 2/27              |
| Hypsugo savii               | nd           | 6/19              |
| Epotesicus serotinus        | nd           | 20/73             |
| Epotesicus isabellinus      | nd           | nd                |
| Plecotus austriacus         | nd           | nd                |
| Nyctalus leisleri           | nd           | nd                |
| *F. Miniopteridae*          |              |                   |
| Miniopterus schreibersii    | 7/63         | 130/842           |
| Miniopterus complex         | nd           | 0/45              |
| *F. Molossidae*             |              |                   |
| Tadarida teniotis           | nd           | 23/105            |
| Total                       | 12/238       | 421/2024          |

|              | Spaina   | Italya  |
|              | SIRb     | Total   |
| F. Rhinolophidae |          |
| Rhinolophus ferrumequinum | 7.90     |
| Rhinolophus euryale     | 6.98     |
| Rhinolophus mehelyi     | 0.00     |
| Rhinolophus blasii     | 0.00     |
| Rhinolophus hipposideros | nd      |
| F. Vespertilionidae     |          |
| Myotis myotis           | nd       |
| Myotis blythii          | nd       |
| Myotis punicus          | 4.92     |
| Myotis capaccinii       | 0.00     |
| Myotis escalerae        | 0.00     |
| Myotis emarginatus      | 0.00     |
| Myotis daubentoni       | nd       |
| Myotis mystacinus       | nd       |
| Pipistrellus pipistrellus | nd      |
| Pipistrellus kuhlii     | nd       |
| Hypsugo savii           | nd       |
| Epotesicus serotinus    | 0.00     |
| Epotesicus isabellinus  | nd       |
| Plecotus austriacus     | nd       |
| Nyctalus leisleri       | nd       |
| F. Miniopteridae        |          |
| Miniopterus schreibersii | 11.11    |
| Miniopterus complex     | 6.80     |
| F. Molossidae           |          |
| Tadarida teniotis       | nd       |
| Total                   | 6.06     |

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*aNumber of seropositive individuals/number of analysed individuals
bPercentage of seropositive individuals per species*
Brain samples were obtained and stored at −80°C. RNA was extracted from brains and tested by nested real-time polymerase chain reaction (nRT-PCR)\textsuperscript{11}.

To our knowledge, this is the first work to report the result of an active lyssavirus survey carried out in bats from Northern Africa. Of the 693 sera analysed, 42 (6%) tested positive for EBLV-1—neutralizing antibodies, and these results were compared to those obtained from two distinct surveys carried out in Spain and Italy\textsuperscript{12,13}. Four of the nine selected locations (44%) in North Africa harbored seropositive bats: one in Morocco and three in Algeria (Table 1). EBLV-1 neutralizing antibodies were detected in five (45%) bat species belonging to three families, and 42 of the 693 (6%) samples were seropositive (Table 1, Supporting Information S1).

Three of the seven bat species analyzed in Morocco were seropositive. \textit{Rhinolophus ferrumequinum} was the Moroccan species with the highest percentage of seropositive individuals (12%). Four of the seven bat species sampled in Algeria tested seropositive, and \textit{M. schreibersii} was the species which had the highest percentage of seropositive individuals (11%) (Table 1). When we compared these percentages with those generated from the studies carried out in southern Europe, we observed that 14 out of 17 (82%) Spanish bats and three out of seven bat species (43%) tested in Italy were positive for EBLV-1. The bat species with the highest percentage of seropositive samples were \textit{M. myotis} and \textit{M. blythii}, respectively (we considered bat species with a sample size higher than 30 individuals). In Europe, 472 (18%) of the 2609 sera tested were positive (seroprevalence by species ranged from 7.41 to 50%).

In addition, we analyzed 19 brains of bats (11 of \textit{R. ferrumequinum}, 1 of \textit{Rhinolophus euryale}, 6 of \textit{Miniopterus} complex and 1 of \textit{M. punicus}) from three different sites on the Atlas region of Morocco. All samples tested negative for lyssavirus RNA.

The specificity of our RFFIT method had already been demonstrated in a previous study\textsuperscript{14} in which a selection of sera from five bat species using the EBLV-1, EBLV-2, and CVS strains had been tested. All sera were positive against EBLV-1 but negative for CVS and EBLV-2; furthermore, the presence of EBLV-1 seropositive bats in Spain, Italy, Morocco and Algeria provides evidence that EBLV-1 may be circulating on both shores of the Mediterranean Sea. Unfortunately, all samples tested by nRT-PCR were negative, which is not surprising considering the relatively reduced number of samples collected and the low expected prevalence of such viruses in bats. While there is no evidence of bat migration between Africa and Europe, virus transmission could be ascribed to the capacity of bats to fly very long distances. Indeed, \textit{M. schreibersii} seems to play an important role in the maintenance of EBLV-1 in some bat colonies of the Balearic Islands\textsuperscript{14} and in Catalonia\textsuperscript{15}.

In the Mediterranean regions, \textit{M. schreibersii} is highly abundant and very likely contributes to virus dispersion\textsuperscript{12}, as it is able to fly and easily cover the distance across the Gibraltar strait (14 km). Similarly, Sicily is the Italian island where seropositive bats were sampled, and its distance from the Tunisian coastline is approximately 142 km. Menorca and Mallorca (islands belonging to the Balearic Islands, Spain) are approximately 170 km from the east coast of the Iberian Peninsula and 270 km from the coast of Algeria. Therefore, further studies are needed to better understand the link between lyssaviruses circulating in Europe and the serological results observed in North Africa.

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Author contributions
Conceived and designed the project: JS-C, HB, CB. Performed fieldwork: ML-R, EA, MEH, AAS, DS, SGER, HYH, AHZ, BH, SEA, BW, MAN, AElZ JS-C. Performed the analysis: HB, RL, PDB, FM, BZ. Wrote the paper: JS-C, HB, ML-R, CB, PDB.

Conflict of interest
The authors declare that they have no conflict of interest.
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References
1. Olival, K. J. et al. Erratum: host and viral traits predict zoonotic spillover from mammals. Nature 548, 612–612 (2017).
2. Schatz, J. et al. Bat rabies surveillance in Europe. Zoonoses Public Health 60, 22–34 (2012).
3. The International Committee on Taxonomy of Viruses Report. (2018). Available: http://ictvonline.org/virusTaxonomy.asp. Accessed on Dec 11, 2018.
4. Nokrèki, T., Tamminranta, N., Kokkonen, U.-M., Kantala, T. & Gadd, T. Tentative novel lyssavirus in a bat in Finland. Transbound Emerg. Dis. 65, 593–596 (2018).
5. Banyard, A. C. & Fooks, A. R. The impact of novel lyssavirus discovery. Microbiol. 38, 17–21 (2017).
6. Kuzmin, I. V. et al. Possible emergence of West Caucasian bat virus in Africa. Emerg. Infect. Dis. 14, 1887–1889 (2008).
7. Milade, J. et al. Serological evidence of lyssaviruses among bats on Southwestern Indian Ocean Islands. PLoS ONE 11, e0160553 (2016).
8. Castella, V. et al. Is the Gibraltar Strait a barrier to gene flow for the bat Myotis myotis (Chiroptera: Vespertilionidae)? Mol. Ecol. 9, 1761–1772 (2000).
9. Amengual, B., López-Roig, M. & Serra-Cobo, J. First record of seasonal over sea migration of Miniopterus schreibersii and Myotis capaccini between Balearic Islands (Spain). Acta Chiropt. 9, 319–322 (2007).
10. Puechmaille, S. J. et al. A new species of the Miniopterus schreibersii species complex (Chiroptera: Miniopteridae) from the Maghreb Region, North Africa. Zootaxa 3794, 108–124 (2014).
11. Serra-Cobo, J., Amengual, B., Abellán, C. & Bourhy, H. European bat Lyssavirus infection in Spanish bat populations. Emerg. Infect. Dis. 8, 413–420 (2002).
12. Serra-Cobo, J. et al. Ecological factors associated with European bat lyssavirus seroprevalence in Spanish bats. PLoS ONE 8, e64467 (2013).
13. Leopardi, S. et al. Active and passive surveillance for bat lyssaviruses in Italy revealed serological evidence for their circulation in three bat species. Epidemiol. Mol. Infect. 1–6 (2018).
14. Pons-Salort, M. et al. Insights into persistence mechanisms of a zoonotic virus in bat colonies using a multispecies metapopulation model. PLoS ONE 9, e95610 (2014).
15. Amengual, B., Bourhy, H., López-Roig, M. & Serra-Cobo, J. Temporal dynamics of European bat Lyssavirus type 1 and survival of Myotis myotis bats in natural colonies. PLoS ONE 2, e566 (2007).