



HAL
open science

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

Jordi Serra-Cobo, Marc López-Roig, Rachel Lavenir, Elbia Abdelatif, Wahida Boucekkine, Mehdi Elharrak, Bachir Harif, Sehhar El Ayachi, Ahmed Salama, Sehhar Hassan, et al.

► To cite this version:

Jordi Serra-Cobo, Marc López-Roig, Rachel Lavenir, Elbia Abdelatif, Wahida Boucekkine, et al.. Active sero-survey for European bat lyssavirus type-1 circulation in North African insectivorous bats. *Emerging microbes & infections*, 2018, 7 (1), pp.213. 10.1038/s41426-018-0214-y . pasteur-01984924

HAL Id: pasteur-01984924

<https://riip.hal.science/pasteur-01984924>

Submitted on 17 Jan 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution| 4.0 International License

CORRESPONDENCE

Open Access

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

Jordi Serra-Cobo^{1,2}, Marc López-Roig^{1,2}, Rachel Lavenir³, Elbia Abdelatif⁴, Wahida Boucekkine⁵, Mehdi Elharrak⁶, Bachir Hari⁶, Sehhar El Ayachi⁷, Akram Ahmed Salama⁸, Mohamed A. Nayel⁸, Ahmed Elsify⁸, Sameh G. El Rashedy⁹, Paola De Benedictis¹⁰, Franco Mutinelli¹⁰, Barbara Zecchin¹⁰, Dino Scaravelli^{11,12}, Chokri Balhoul¹³, Ahmed Zaghawa⁸, Hany Youssef Hassan⁸, Ahmed Hamed Zaghloul¹⁴ and Hervé Bourhy³

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 mammals¹. Among these zoonotic viruses, lyssaviruses are known to circulate among the European insectivorous bats². 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* (WCBV) and the tentative novel member of the genus *Lyssavirus Kotalahti* bat lyssavirus^{3,4}.

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 *Shimoni bat virus* (SHIBV), isolated from *Hipposideros vittatus* in Kenya (2009)⁵. However, serological evidence of *West Caucasian bat virus* (WCBV) and EBLV-1 infection has been reported in bats from Kenya⁶ and southwestern Indian Ocean islands⁷, 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 Sea⁸, migratory species such as *Miniopterus schreibersii* can fly over the sea for long distances⁹. 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 maghrebensis* lives. This bat species was described only after our fieldwork had been completed¹⁰, which means that we had no way to retrospectively determine which individuals belonged to the

Correspondence: Jordi Serra-Cobo (serracobo@areambiental.com)

¹IRBIO and Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain

²Centre de Recerca en Infeccions Virals, Illes Balears (CRIMB), Fundació d'Investigació Sanitària de les Illes Balears, Conselleria de Salut i Consum, Govern de les Illes Balears, Hospital General de Palma, 07012 Palma de Mallorca, Illes Balears, Spain

Full list of author information is available at the end of the article.

© The Author(s) 2018



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

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 | | | Total |
|----------------------------------|----------------------|----------------------|------------------|--------------------|--------------------|------------------|-------|
| | Algeria ^a | Morocco ^a | SIR ^b | Spain ^a | Italy ^a | SIR ^b | |
| <i>F. Rhinolophidae</i> | | | | | | | |
| <i>Rhinolophus ferrumequinum</i> | 1/47 | 8/67 | 7.90 | 38/165 | 0/15 | 20.54 | 15.71 |
| <i>Rhinolophus euryale</i> | 3/31 | 0/12 | 6.98 | 1/2 | nd | 50.00 | 8.89 |
| <i>Rhinolophus mehelyi</i> | nd | 0/7 | 0.00 | nd | nd | nd | 0.00 |
| <i>Rhinolophus blasii</i> | 0/1 | nd | 0.00 | nd | nd | nd | 0.00 |
| <i>Rhinolophus hipposideros</i> | nd | nd | nd | nd | 0/5 | 0.00 | 0.00 |
| <i>F. Vespertilionidae</i> | | | | | | | |
| <i>Myotis myotis</i> | nd | nd | nd | 127/390 | 16/205 | 24.03 | 24.03 |
| <i>Myotis blythii</i> | nd | nd | nd | 18/105 | 11/52 | 18.47 | 18.47 |
| <i>Myotis punicus</i> | 1/63 | 15/262 | 4.92 | nd | nd | nd | 4.92 |
| <i>Myotis capaccinii</i> | 0/6 | nd | 0.00 | 17/99 | 0/9 | 15.74 | 14.91 |
| <i>Myotis escaleraei</i> | nd | 0/1 | 0.00 | 6/18 | nd | 33.33 | 31.58 |
| <i>Myotis emarginatus</i> | 0/27 | nd | 0.00 | 0/2 | nd | 0.00 | 0.00 |
| <i>Myotis daubentonii</i> | nd | nd | nd | 7/54 | nd | 12.96 | 12.96 |
| <i>Myotis mystacinus</i> | nd | nd | nd | 0/1 | nd | 0.00 | 0.00 |
| <i>Pipistrellus pipistrellus</i> | nd | nd | nd | 7/39 | nd | 17.95 | 17.95 |
| <i>Pipistrellus kuhlii</i> | nd | nd | 0.00 | 2/27 | nd | 7.41 | 6.90 |
| <i>Hypsugo savii</i> | nd | nd | nd | 6/19 | nd | 31.58 | 31.58 |
| <i>Eptesicus serotinus</i> | nd | nd | nd | 20/73 | nd | 27.40 | 27.40 |
| <i>Eptesicus isabellinus</i> | nd | 0/3 | 0.00 | nd | nd | nd | 0.00 |
| <i>Plecotus austriacus</i> | nd | nd | nd | 19/74 | nd | 25.67 | 25.67 |
| <i>Nyctalus leisleri</i> | nd | nd | nd | 0/9 | nd | 0.00 | 0.00 |
| <i>F. Miniopteridae</i> | | | | | | | |
| <i>Miniopterus schreibersii</i> | 7/63 | nd | 11.11 | 130/842 | 0/45 | 14.66 | 14.42 |
| <i>Miniopterus complex</i> | nd | 7/103 | 6.80 | nd | nd | nd | 6.80 |
| <i>F. Molossidae</i> | | | | | | | |
| <i>Tadarida teniotis</i> | nd | nd | nd | 23/105 | 24/254 | 13.09 | 13.09 |
| Total | 12/238 | 30/455 | 6.06 | 421/2024 | 51/585 | 18.09 | 15.56 |

^aNumber of seropositive individuals/number of analysed individuals^bPercentage of seropositive individuals per species

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.

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)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^{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. *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 *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 *M. myotis* and *M. blythii*, respectively (we considered bat species with a sample size higher 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 *R. ferrumequinum*, 1 of *Rhinolophus euryale*, 6 of *Miniopterus* complex and 1 of *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 study¹¹ 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, *M. schreibersii* seems to play an important role in the maintenance of EBLV-1 in some

bat colonies of the Balearic Islands¹⁴ and in Catalonia¹⁵. In the Mediterranean regions, *M. schreibersii* is highly abundant and very likely contributes to virus dispersion¹², 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.

Acknowledgements

We wish to thank the National Park of Aiguestortes i Estany de SantMaurici (Lleida, Spain), the Conselleria de Medi Ambient, Agricultura i Pesca (Govern de les Illes Balears, Spain), the Natural Park of Sant Llorenç del Munt i l'Obac (Barcelona, Spain), the Malgrat de Mar City Council, the National Park of Chréa (Algeria), the National Park of Béjaia (Algeria) and Dr. Ahmim Mourad for their precious the contribution during field work. This work was funded by the EU FP6 program, Project RABMEDCONTROL, (<http://www.rabmedcontrol.org>) and FP7 for research, technological development and demonstration under Grant Agreement No. 278433 PREDEMICS ("Preparedness, Prediction and Prevention of Emerging Zoonotic Viruses with Pandemic Potential using Multidisciplinary Approaches").

Author details

¹IRBIO and Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain. ²Centre de Recerca en Infeccions Viriques, Illes Balears (CRIMB), Fundació d'Investigació Sanitària de les Illes Balears, Conselleria de Salut i Consum, Govern de les Illes Balears, Hospital General de Palma, 07012 Palma de Mallorca, Illes Balears, Spain. ³Institut Pasteur, Unité Dynamique des Lyssavirus et Adaptation à l'Hôte, WHO Collaborating Centre for Reference and Research on Rabies, Paris Cedex 15, 75724 Paris, France. ⁴Institut Pasteur d'Alger, 16047 Dély Ibrahim, Alger, Algeria. ⁵Direction Générale des Forêts, Chemin Doudou Mokhtar Ben-Aknoun, B.P.232, 16306 Alger, Algeria. ⁶Société de Produits biologiques et pharmaceutiques et vétérinaires (Biopharma), Km 2, Road of Casablanca, B.P, 4569 Rabat-Akkari, Morocco. ⁷Département Ressources Naturelles et Environnement Institut Agronomique et Vétérinaire Hassan II, Madinat Al Irfane, B.P, 6202 Rabat, Morocco. ⁸Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, 32897 Sadat City, Minoufiya, Egypt. ⁹Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, University of Sadat City, 32897 Sadat City, Minoufiya, Egypt. ¹⁰FAO and National Reference Centre for rabies & OIE Collaborating Centre for diseases at the animal-human interface, Division of Biomedical Science, Istituto Zooprofilattico Sperimentale delle Venezie, 35020 Legnaro, Italy. ¹¹S.T.E.R.N.A. & Museo Ornitologico "F. Foschi", 47121 Forlì, Italy. ¹²Laboratory of Pathogens' Ecology, Department of Veterinary Medical Sciences, University of Bologna, 40064 Ozzano Emilia (Bo), Italy. ¹³Institut Pasteur Tunis, Place Pasteur B.P. 74, 1002 Tunis, Belvédère, Tunisia. ¹⁴Department of Theriogenology and Artificial Insemination, Faculty of Veterinary Medicine, University of Sadat City, 32897 Sadat City, Minoufiya, Egypt

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, AEIZ 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.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information accompanies this paper at (<https://doi.org/10.1038/s41426-018-0214-y>).

Received: 2 July 2018 Revised: 18 October 2018 Accepted: 20 November 2018

Published online: 13 December 2018

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. Nokireki, T., Tammiranta, 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. Austral* **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. Mélade, J. et al. Serological evidence of lyssaviruses among bats on South-western 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 capaccinii* between Balearic Islands (Spain). *Acta Chiropt.* **9**, 319–322 (2007).
10. Puechmaile, 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. 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).