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ORIGINAL ARTICLE

# Surveillance for European bat lyssavirus in Swiss bats

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**Abstract** Most countries in Western Europe are currently free of rabies in terrestrial mammals. Nevertheless, rabies remains a residual risk to public health due to the natural circulation of bat-specific viruses, such as European bat lyssaviruses (EBLVs). European bat lyssavirus types 1 and 2 (EBLV-1 and EBLV-2) are widely distributed throughout Europe, but little is known of their true prevalence and epidemiology. We report that only three out of 837 brains taken from bats submitted to the Swiss Rabies Centre between 1976 and 2009 were found by immunofluorescence (FAT) to be positive for EBLVs. All three positive cases were in *Myotis daubentoni*, from 1992, 1993 and 2002. In addition to this passive surveillance, we undertook a targeted survey in 2009, aimed at detecting lyssaviruses in live bats in Switzerland. A total of 237 bats of the species *M. daubentoni*, *Myotis myotis*, *Eptesicus serotinus* and *Nyctalus noctula* were captured at different sites in western Switzerland. Oropharyngeal swabs and blood from each individual were analysed by RT-PCR and rapid fluorescent focus inhibition test (RFFIT), respectively. RNA corresponding to EBLV-2 was detected from oropharyngeal swabs of a single *M. daubentoni* bat, but no infectious virus was found. Molecular phylogenetic analysis revealed that the corresponding sequence was closely related to the other EBLV-2 sequences identified in previous rabies isolates from Swiss bats (particularly to that found at Geneva in 2002). Three *M. daubentoni* bats were found to be seropositive by RFFIT. In conclusion, even though the prevalence is low in Switzerland, continuous management and surveillance are required to assess the potential risk to public health.

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## Introduction

Bats across the world have been recognized as reservoir hosts for a number of emerging infectious diseases and zoonoses that can cross the species barriers and infect other mammals, including humans (reviewed in [1, 2]). Rabies is the oldest recorded zoonosis, and still the most important one using bats as a reservoir host [3].

Rabies is a worldwide viral zoonosis that can infect all mammals, including humans (for a recent review, see ref. [4]). The infection is due to lyssaviruses (genus *Lyssavirus*, family *Rhabdoviridae*), and several species of carnivore, apart from bats, act as reservoirs. There are currently 11 recognized species [5–7] and one newly described genotype in the genus *Lyssavirus* [8], including the European bat lyssavirus type 1 (EBLV-1, genotype 5) and the European bat lyssavirus type 2 (EBLV-2; genotype 6). All but one genotype (Mokola virus) have been found in bats

[9]. The first rabid insectivorous bat in Europe was recorded in Hamburg, Germany, in 1954 [10]. Over 850 cases of rabies-positive bats have subsequently been recorded between 1977 and 2008 [11]. Most of the cases were EBLV-1, and 95% of all reported EBLV-1 infections were found in serotine bats, *Eptesicus serotinus* [12, 13]. By contrast, EBLV-2 has been discovered only 20 times to date, mostly in Daubenton's bats, *Myotis daubentoni*, in Finland [14], Switzerland [15], the United Kingdom [16, 17] and Germany [18] and in pond bats, *Myotis dasycneme*, in the Netherlands [12]. So far, the Netherlands and Germany are the only countries where both genotypes have been isolated from rabies-positive bats [12, 18].

EBLVs are of public health concern, as both EBLV-1 and EBLV-2 have been transmitted to humans and resulted in fatalities (for a recent review, see ref. [4]). In 1985, a bat researcher in Finland died of rabies encephalitis caused by EBLV-2b [19]. However, the geographical location of the infection could not be pinpointed because this researcher had handled bats in several countries, including Switzerland, during the previous years. In a targeted surveillance, undertaken in 1986 in southern Finland, no lyssavirus infection could be detected from a sample of 183 bats [20]. The first EBLV-2-positive Daubenton's bat in Finland was finally reported in 2009 [14]. Phylogenetic analysis showed that the EBLV-2b isolate from the human case in 1985 was most closely related to the recent Finnish bat isolate, indicating that the source of the human infection might have been in Finland (see [14]).

However, our knowledge on prevalence epidemiology and pathogenesis of EBLVs remains partial and enigmatic. Clinically silent rabies infections have been described in several bat species worldwide [21–23]. Viral RNA has been detected in the saliva, blood and various organs of healthy bats captured in field colonies. The excretion of viral RNA in the saliva may persist and fluctuate over time [24]. Similarly, antiviral antibodies were also detected in healthy captured bats. On the other hand, most cases detected so far have been in diseased or dead animals, and experimental infection of bats usually leads to short excretion and fast death [22, 25]. Nevertheless, the risk of rabies transmission to humans is real and raises important implications for bat management and bat specialists protection [26].

Herein, we present a compilation of the passive surveillance conducted so far and the results of a recent targeted survey in four bat species living in western Switzerland. In the targeted survey, we focused on *M. daubentoni*, *M. myotis* and *E. serotinus*, as members of these three species have been found to be infected with rabies virus in European countries, and on *Nyctalus noctula*, as this species migrates over long distances [27] and could potentially disseminate the disease over a large geographical range while infected. *Myotis dasycneme* is also a potential host for EBLV-2, but the species is not found in Switzerland.

## Materials and methods

### Sample collection

#### *(a) Passive surveillance*

Passive surveillance for EBLVs, based on dead and diseased animals with clinical suspicion, was initiated in Switzerland in 1966 by the Swiss Rabies Centre. Dead and freshly euthanized animals were submitted to the Swiss Rabies Centre by bat conservationists, museum curators, veterinarians or the general public.

#### *(b) Active surveillance*

Bats (*M. daubentoni*, *M. myotis*, *E. serotinus* and *N. noctula*) were captured in 2009 at 12 different locations around Lake Geneva and close to Lake Neuchâtel in the cantons of Vaud, Geneva and Fribourg, in Switzerland. Greater mouse-eared bats were captured either inside reproductive colonies ($n = 40$) or at the entrance of caves, noctule bats inside pre-hibernating colonies (nest boxes), serotine bats at the entrance of caves and Daubenton's bats while flying over rivers using mist nests or harptraps. The sex, reproductive status, age, body mass, forearm length and general body condition were recorded. Signs of poor health, such as poor condition of fur, state of emaciation, abnormal behaviour, parasitic load and excessively injured wings (rips, tears and punctures) were also recorded. A uniquely numbered bat ring (Museum of Natural History, Geneva, Switzerland) was fitted on each bat for future individual recognition. Capturing, handling, ringing and sampling of bats were done under the current laws of Switzerland (Authorization of Consumer and Veterinary Services of Canton of Vaud no. 2203, Switzerland).

Two oropharyngeal swabs (one dry, one wet) were taken from each bat to collect saliva in order to determine the presence of EBLV. The swabs were then immediately placed on dry ice (4°C) and brought back to the laboratory. All swabs were stored at −80°C until analysis.

Blood samples were taken with a 0.55 × 25 mm needle (Romedic SA, Lausanne, Switzerland) from the uropatagial vein and collected in a Microvette CB300 capillary tube (Sarstedt, Nümbrecht, Germany) with EDTA. The volume of blood sampled varied between 5 and 100 µl. Haemostatic cotton (Flawa, Flawil, Switzerland) was then applied to the puncture site to prevent further bleeding and to facilitate healing. The bat was then kept for about 15 min

after taking a blood sample to let it calm down. The health condition of each bat was assessed before its release. Blood samples were stored at ~2°C in the field for a few hours. In the laboratory, samples were centrifuged at 1,400×*g* for 15 min, and the plasma was stored at −80°C until analysis.

## Sample analysis

### *(a) Detection of EBLV antigens*

The standard fluorescent antibody test (FAT) was performed on the brain tissues of all individuals from the passive surveillance programme using a previously described method [28].

### *(b) Isolation of infectious virus*

Virus isolation was performed using a rabies tissue-culture infection test (RTCIT) with four consecutive passages in neuroblastoma cells (MNA 42/13, kindly provided by Dr. W. Müller, Tübingen, 1994) inoculated with 20% brain or saliva suspension, followed by immunofluorescence staining for rabies virus after each passage [29, 30].

### *(c) Detection of EBLV RNA*

All saliva samples were analysed by RT-PCR to detect the presence of host and viral RNA. Five hundred microlitres of RNA Storage Solution (Ambion, Foster City, CA, USA) was added to either the wet or the dry frozen swab of each of the 237 bats sampled. The swabs were then thawed for 10 min at room temperature, vortexed vigorously for 1 min, and then centrifuged at 5,000 rpm for 10 min. RNA was extracted from 140 µl of the supernatant using a QIAmp Viral RNA Kit (QIAgen, Germantown, MD, USA). To detect viral RNA, a reverse transcription PCR (RT-PCR) was performed using a OneStep RT-PCR Kit (QIAgen, Germantown, MD, USA), following a protocol adapted from Vazquez-Moron et al. [31]. The primers GRAB1F and GRAB1R, designed by Vazquez-Moron et al. [31], bind to a conserved region of the nucleoprotein gene of the first seven recognized lyssavirus genotypes (260 bp). Nested PCR was performed using the primers GRAB2F and GRAB2R [31]. All amplifications were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). PCR products were visualised under UV light after gel electrophoresis in 1.5% agarose.

The interpretation of PCR results assumed that each swab contained host material (host-derived RNA) from the oral cavity. In order to prevent false negative detection due to absence of host material, a second RT-PCR was conducted on each sample, using specific primers that bind to the mammalian glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene according to Ravazzolo et al. [32]. The RT-PCR reaction was conducted in a total volume of 50 µl, using a OneStep RT-PCR Kit (QIAgen, Germantown, MD, USA) as described above for the first round of nested PCR. PCR products were run on ethidium-bromide-stained polyacrylamide gels and visualized under UV light. GAPDH-positive samples showed a specific band at 125 bp.

Positive (CVS) and negative (RNA Storage Solution or $H_2O$) controls were added at each of the following steps: RNA extraction, RT-PCR, and nested PCR. When a saliva sample was found to be positive, the second corresponding saliva sample from that bat was also tested following the procedure described above.

### *(d) Detection of EBLV antibodies*

A microtitre adaptation of RFFIT in 96-well microtitre tissue culture trays was performed essentially as described [33, 34]. The samples were serially diluted from 1:25 to 1:625 in BHK-21 cell culture medium. A pool of human sera calibrated with the second International Standard Preparation for Rabies Immunoglobulins was used as a control standard [35]. CVS-11 (Challenge Virus Strain; [36]) or EBLV-2 virus was used as challenge virus. The virus-neutralizing antibody (VNA) titres were calculated according to Spearman and Kaerber [37, 38] by extrapolating the dilution of the sample that reduces the number of fluorescent microscopic fields to 50%. International units (IU) were determined using a standard control (9.0 IU/ml).

## Phylogenetic reconstruction

The 220-bp nucleotide sequences of the nucleoprotein gene obtained in this study were edited using MEGA v 4.0 [39], aligned using CLUSTAL 2.0.12 [40], visually inspected and manually corrected. Additional sequences were retrieved from GenBank (see Table 1).

Phylogenetic trees were constructed using two different methods: maximum likelihood (ML) and Bayesian analysis (BA). The model of DNA substitution was selected using jModeltest v 0.1.1 [41] and the minimum Akaike information criterion value. The HKY85 + G model best fitted the data. ML heuristic searches and bootstrap analyses (1,000 replicates) were performed using PHYML v 3.0 [42]. The BA was performed with MrBayes v 3.1.2 [43, 44]. Two independent runs were performed, each of them consisting of four parallel MCMC chains of five million generations, allowing a good convergence of the independent runs. After having visualised the parameters and controlled for a burn-in period using the software TRACER v 1.5 [45], 10% of the trees were discarded as burn-in period. The remaining trees were used to construct a 50% major-consensus tree.

**Table 1** The site of collection, year of isolation, bat species and GenBank accession number for each EBLV strain used in the phylogenetic analyses

| Virus | Year of isolation | Locality | Country | Bat species | Accession no. |
|---|---|---|---|---|---|
| EBLV-2 | | | | | |
| 70 | 2009 | Genthod (GE) | Switzerland | *M.daubentoni* | HM067110[a] |
| 118 | 2002 | Geneva (GE) | Switzerland | *M.daubentoni* | HM067109[a] |
| RV1333 | 2002 | NA | United Kingdom | *M.daubentoni* | EF157977 |
| 1392 | 1993 | Versoix (GE) | Switzerland | *M.daubentoni* | HM067108[a] |
| 9375 | 1993 | Roden | The Netherlands | *M.dasycneme* | AY863404 |
| 1814 | 1992 | Plaffeien (FR) | Switzerland | *M.daubentoni* | HM067107[a] |
| 94112 | 1989 | Andijk | The Netherlands | *M.dasycneme* | AY863405 |
| 9018 | 1987 | Wommels | The Netherlands | *M.dasycneme* | AY863403 |
| 9007 | 1986 | Helsinki | Finland | Human | AY863406 |
| EBLV-1 | | | | | |
| 102 | 2000 | Haute-Marne | France | *E.serotinus* | AY863399 |
| 9477 | 1986 | Niedersachsen | Germany | *E.serotinus* | AY863357 |

*NA* information not available

[a] This study

## Results

### Passive surveillance and detection of EBLV antigens

A total of 837 bat brain specimens were analysed in FAT/RTCIT between 1976 and 2009 (Table 2). Daubenton's bat was the only species found to be positive for lyssavirus infection. Three cases of EBLV-2b have been identified, one on August 24, 1992, in Plaffeien, canton of Fribourg, one on July 10, 1993, in Versoix, canton of Geneva, and one on September 2, 2002, in the city of Geneva, canton of Geneva. No bat infected with EBLV-1 has been recorded. The numbers of bats submitted annually varied from 0 to 5 from 1976 to 1985 to 11 to 90 since 1986. Almost 50% of the submitted specimens were pipistrelles (*Pipistrellus* spp.). Notably, only 64 bats were *M. daubentoni* (7.6%), and 21 were *Eptesicus serotinus* (2.5%), which are the two main vectors of EBLVs. The surveillance intensity was regionally biased, with significantly higher numbers of submissions from large agglomerations than from the countryside, e.g. the area around the first Swiss case in the canton of Fribourg (Fig. 1). Overall, the area covered roughly corresponds to the most suitable habitats for bats in Switzerland, avoiding higher mountainous regions.

**Table 2** The number of individuals and the percentage of each species (%) for all passive surveillance bat submissions from 1976 to 2009

| Species | *n* submitted | % | Rabies positive |
|---|---|---|---|
| *Barbastella barbastellus* | 3 | 0.4 | 0 |
| *Eptesicus nilssoni* | 13 | 1.6 | 0 |
| *Eptesicus serotinus* | 21 | 2.5 | 0 |
| *Hypsugo savii* | 1 | 0.1 | 0 |
| *Myotis bechsteini* | 5 | 0.6 | 0 |
| *Myotis blythii* | 2 | 0.2 | 0 |
| *Myotis daubentoni* | 64 | 7.6 | 3 |
| *Myotis myotis* | 41 | 4.9 | 0 |
| *Myotis mystacinus* | 45 | 5.4 | 0 |
| *Myotis nattereri* | 4 | 0.5 | 0 |
| *Myotis sp.* | 1 | 0.1 | 0 |
| *Nyctalus leisleri* | 21 | 2.5 | 0 |
| *Nyctalus noctula* | 68 | 8.1 | 0 |
| *Pipistrellus kuhli* | 57 | 6.8 | 0 |
| *Pipistrellus nathusii* | 112 | 13.4 | 0 |
| *Pipistrellus pipistrellus* | 224 | 26.8 | 0 |
| *Pipistrellus pygmaeus* | 11 | 1.3 | 0 |
| *Pipistrellus savii* | 2 | 0.2 | 0 |
| *Plecotus auritus* | 63 | 7.5 | 0 |
| *Plecotus austriacus* | 5 | 0.6 | 0 |
| *Plecotus macrobullaris* | 2 | 0.2 | 0 |
| *Plecotus sp.* | 3 | 0.4 | 0 |
| *Pipistrellus sp.* | 24 | 2.9 | 0 |
| *Vespertilio murinus* | 31 | 3.7 | 0 |
| Unspecified | 14 | 1.7 | |
| Total | 837 | 100 | 3 |

### Detection of EBLV antibodies

A minimum volume of 5 µl of plasma is necessary to proceed with RFFIT. Thus, only 202 bats out of the 237 sampled were subjected to the analysis (124/148 *M. daubentoni*, 47/51 *M. myotis*, 17/23 *E. serotinus* and 14/15 *N. noctula*; Table 3). All samples were tested individually for antibody with CVS and/or EBLV-2b as challenge virus.

One hundred fifty specimens (109 *M. daubentoni*, 24 *M. myotis*, 10 *E. serotinus* and 7 *N. noctula*) were tested in the assay with CVS as the challenge virus, out of which 130 were tested at initial dilutions of 1:25, and 20 at 1:125.

One hundred sixty samples, including 94 *M. daubentoni*, 46 *M. myotis*, 11 *E. serotinus* and 9 *N. noctula*, were also

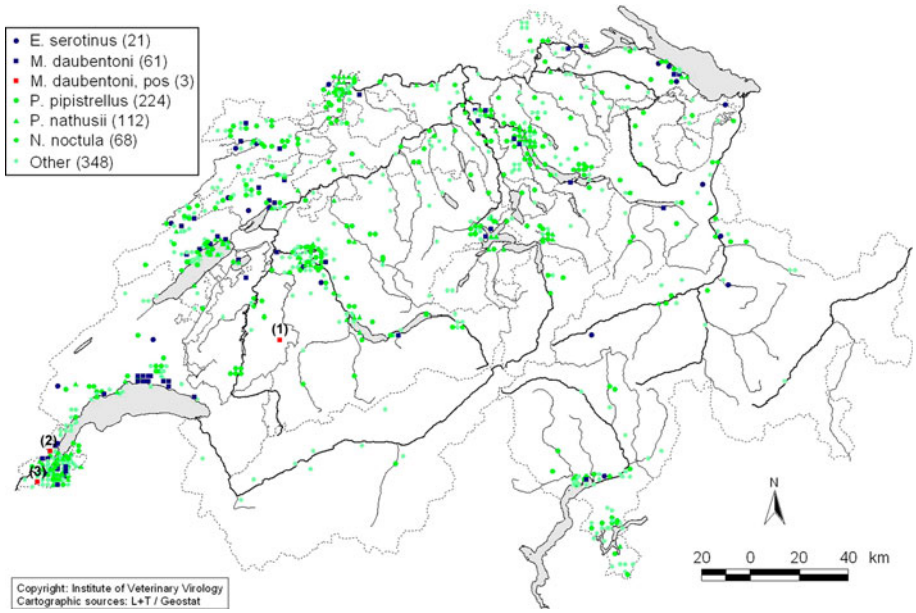


**Fig. 1** The distribution of records across Switzerland from passive bat rabies surveillance (1967–2009). The most frequently submitted species and the positive cases are indicated (including the two major host species *E. serotinus* and *M. daubentoni*). The coordinates of individual samples were shifted for clarity. *1* August 24, 1992, *M. daubentoni*, Plaffeien, Fribourg, *2* July 10, 1993, *M. daubentoni*, Versoix, Geneva, *3* September 2, 2002, *M. daubentoni*, City of Geneva, Geneva

tested with EBLV-2b as the challenge virus (previously isolated from one of the three positive Swiss Daubenton's bats, ID 1392/93), out of which 126 were tested at an initial dilution of 1:25 and 35 at an initial dilution of 1:125.

Three bats, all Daubenton's bats from different sampling sites, were found to be seropositive. One specimen sampled in Baulmes, Vaud, was positive with CVS (titre 1:56) but could not be tested at the same initial dilution with EBLV-2b (negative at 1:125 dilution). The other two seropositive bats were sampled in Dorigny, Vaud (titre 1:70), and in Genthod, Geneva (titre 1:314), and were found positive with EBLV-2b but not with CVS as challenge virus. Following previous suggestions, we used a reciprocal titre of $\geq$27 as the positive threshold [46].

### Detection of EBLV RNA

A total of 237 bats, including 148 *M. daubentoni*, 51 *M. myotis*, 23 *E. serotinus* and 15 *N. noctula*, were analysed. Host GAPDH was detected in all of the swabs analysed, indicating that material originating from the sampled bat was present on the swab. No difference in the ability to detect RNA in the wet or dry swabs was observed. Both methods are therefore suitable to swab bats. Of the 237 bats sampled, only one Daubenton's bat (0.4%) was found positive for EBLV-2b RNA by the nested PCR approach, using both wet and dry swabs. Virus isolation over four passages using RTCIT was negative with both swabs. This bat was an adult female caught in Genthod in the canton of Geneva. It was found to host nine parasitic wing mites (*Spinturnix andegavinus*) and did not show any signs of poor health or unusual behaviour.

**Table 3** The number of individuals sampled around Lake Geneva and close to Lake Neuchâtel, tested serologically (CVS/EBLVs) and by RT-PCR for rabies, per site and per species of bats

| Species | *n* sites | *n* sampled | *n* RFITT/+ | *n* RT-PCR/+ |
|---|---|---|---|---|
| *M. daubentoni* | 9 | 148 | 124/3 | 148/1 |
| *M. myotis* | 4 | 51 | 47/0 | 51/0 |
| *N. noctula* | 2 | 15 | 14/0 | 15/0 |
| *E. serotinus* | 1 | 23 | 17/0 | 23/0 |
| Total | | 237 | 202/3 | 237/1 |

### Phylogenetic reconstruction

The two phylogenetic methods used, ML and BA, resulted in similar tree topologies. The resulting phylogeny obtained by BA analysis is presented in Fig. 2. The sequence of the Daubenton's bat sample (Genthod, canton of Geneva) that was found to be positive in RT-PCR (DEE70.SWI) grouped with the other three EBLV-2b sequences identified previously in Swiss Daubenton's bats, particularly with the one that was found in the city of Geneva in 2002 (118.SWI). The bootstrap support for the subdivision of EBLV-2 in the lineages EBLV-2a and EBLV-2b as suggested before [24] is reasonable (0.91 in BA) but not overwhelming.

## Discussion

The present study confirms the presence of EBLV-2 and extends our knowledge about its epidemiology in the Swiss bat population. The pattern of passive surveillance showed clear drawbacks, both with respect to spatial distribution

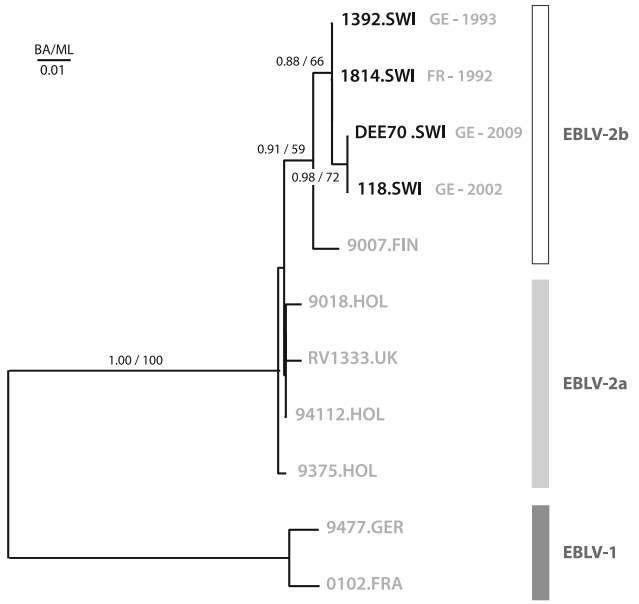


**Fig. 2** Consensus Bayesian phylogenetic tree (50% majority consensus) and branch length for partial nucleoprotein gene sequences from GenBank, depicting the relationships of the Swiss isolates to other isolates. The tree is rooted with two *EBLV-1* samples. The results are based on two runs of $5 \times 10^6$ generations, each with 4 chains

and the species representation of the main vectors *E. serotinus* and *M. daubentoni*. During the passive surveillance, three *M. daubentoni* were found to be positive for EBLV-2, in 1992, 1993 and 2002. During the 4-month targeted surveillance programme, seropositive healthy Daubenton's bats and a PCR-positive saliva of another healthy Daubenton's bat were found. This RT-PCR-positive bat was captured in Genthod, close to Versoix, where the second case of EBLV in Swiss bats was detected in 1993. This sequence is closely related to the corresponding sequences of the other three cases of EBLV-2 diagnosed in Switzerland. Recently, EBVL-2 has also been detected in a Daubenton's bat in Germany [18].

Both the detection of neutralizing antibodies in blood samples, indicating past exposure to EBLV, and the detection of viral RNA in oropharyngeal swabs suggest recent active circulation of EBLV in Swiss Daubenton's bats, confirming the potential for disease spread. Not a single bat from the three other species sampled (Serotine bat, Noctule bat and Great mouse-eared bat) gave a positive reaction by serology or RT-PCR. Out of the three seropositive Daubenton's bats, one was found positive with CVS as challenge virus, probably due to serological cross-reactivity with EBLVs. The other two samples were positive with EBLV-2b but not with CVS, indicating higher sensitivity of the neutralization test when using EBLV as the challenge virus. The seroprevalence over the different Daubenton's bats was 2.4% (3 of 24 bats tested), whereas the prevalence estimated by RT-PCR was lower, with 0.7% of individuals reacting positively (1/148). The low prevalence found in this work seems to be in accordance with the north-to-south gradient in the frequency of EBLVs detected in Western Europe using classical techniques for viral detection [47]. In Scotland, a seroprevalence of 0.05–3.8% has been detected while screening *M. daubentoni* [48]. In a recent active survey in England, a seroprevalence of 1.0–4.1% was found, while no virus was detected using both RT-PCR and RTCIT in 363 *M. daubentoni* bats sampled [46].

EBLV-1 is the most common virus found across northern and central Europe as far east as Russia, and in western European countries as far south as Spain (reviewed in ref. [49]). Germany and the Netherlands are the only countries in which both EBLV-1 and EBLV-2 have been detected. EBLV-1 has a specific association with the serotine bat as the main vector, but it has also been reported in greater mouse-eared bats in Spain [50, 51] and in noctule bats in Germany [52]. Although serotine bats, noctule bats and greater mouse-eared bats are frequently found across Switzerland ([53] and Centre Suisse de Cartographie de la Faune, Neuchâtel), few samples have been analysed (Table 2) and none were found positive in the present study. In view of regular cases encountered in serotine bats both in France and Germany [13, 54], this infection may have been missed in both passive and active surveillance so far due to the limited number of individuals tested and the biased distribution of sampling.

Appreciation of the potential for epidemiological spread and disease risk requires an understanding of host dispersal. Seasonal movements of bats between summer and winter roosts over hundreds of kilometres may provide opportunities for disease spread. A noctule bat ringed in Lausanne, Switzerland, was caught 670 km farther north, near Leipzig, Germany (P. Christe, O. Glaizot, and D. Brockmann, personal communication), and movements up to 1,500 km have been recorded for this species [55]. A recent genetic study revealed a relative genetic uniformity of UK and European continental Daubenton's bat populations, implying that there is no migration barrier to EBLV-2 between regions [56]. Movement of bats over long distances is therefore common and important with respect to the epidemiology of EBLV, indicating a high potential for spread of disease.

## Conclusion

Overall, the results of this study suggest that the public-health risk of infection with EBLV is moderate to low in Switzerland. Nonetheless, the practice of considering rabies in both humans and animals with a course of progressive encephalitis, also in the absence of a known

history of exposure, must be maintained and ensured. The risk of cross-species transmission to both humans and animals is highest in clinically diseased, disoriented animals with bizarre behaviour. EBLV-1 has not been detected in any of the bats sampled, and EBLV-2 seems to be present only at a very low prevalence. However, with the present confirmation of EBLV-2 infection in Daubenton's bats, and considering the biased sampling, the epidemic picture of EBLV in Switzerland remains incomplete. Therefore, both active and passive surveillance must be intensified in order to assess more appropriately the potential risk to public health.

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