

3. Miscellaneous Articles

3. 1 Passive and Targeted Surveillance for European Bat Lyssavirus in Swiss Bats

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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 European bat lyssaviruses (EBLV-1 & EBLV-2), which are widely distributed throughout Europe. Little is known of their true prevalence and epidemiology. In Switzerland, a total of 837 bat brain specimens have been analysed in FAT (fluorescent antibody test)/RTCIT (rabies tissue-culture infection test) between 1976 and 2009. Daubenton's bat was the only species found to be positive, one in August 24, 1992 in Plaffeien, canton of Fribourg, one in July 10, 1993 in Versoix, canton of Geneva and one in September 2, 2002 in the City of Geneva, canton of Geneva. So far, no bat infected with EBLV-1 has been found. The numbers of bats submitted varied from 1 to several dozens since 1986. Almost 50% of the submitted specimens were pipistrelles (*Pipistrellus* spp.) whereas only 64 bats were *M. daubentoni* (7.6%), and 21 were *Eptesicus serotinus* (2.5 %), the two main vectors of EBLVs. Overall, the area covered roughly the most

suitable habitats for bats in Switzerland, avoiding higher mountainous regions.

A total of 237 bats captured at different sites in western Switzerland in 2009, including 148 *M. daubentoni*, 51 *M. myotis*, 23 *E. serotinus* and 15 *N. noctula* were analysed for both viral RNA and antiviral neutralising antibodies (RFFIT, rapid fluorescent focus inhibition test, Smith *et al.*, 1973; Zalan *et al.*, 1979) in oropharyngeal swabs and blood samples, respectively (Fig. 1). Of these, only one Daubenton's bat (0.4%) was found positive for EBLV-2b RNA in RT-PCR using nested primers (Vazquez-Moron *et al.*, 2006). Since virus isolation over four passages using RTCIT was negative, it was not considered a reportable case (Picard *et al.*, 2005). This bat was an adult female caught in Genthod in the canton of Geneva not showing any signs of poor health condition or bizarre behaviour. Phylogenetic reconstruction revealed close relatedness of the sequence of this sample with the other three EBLV-2b sequences previously identified in Swiss Daubenton's bats, particularly to the

one that has been found in Geneva city in 2002. The bootstrap support for the subdivision of EBLV-2 in the lineages EBLV-2a and EBLV-2b as suggested before (Amengual *et al.* 1997) was reasonable (not shown). Furthermore, 202 blood samples of the captured bats (124 *M. daubentoni*, 48 *M. myotis*, 17 *E. serotinus* and 14 *N. noctula*) were tested individually for rabies virus neutralising antibodies using CVS and/or EBLV-2b as challenge virus. Three Daubenton's bats from different sampling sites were found to be seropositive. The positive samples were from Baulmes, canton of Vaud, Dorigny, canton of Vaud and Genthod, canton of Geneva.

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. No positive sample either in serology or RT-PCR was found in the other three species sampled (Serotine bat, Noctule bat and Greater Mouse-eared bat). The seroprevalence in Daubenton's bat was 2.4% (3/124 bats tested), whereas the prevalence estimated by RT-PCR was 0.7% (1/148 bats). This low prevalence seems to be in accordance with the north-to-south gradient in the frequency of EBLVs

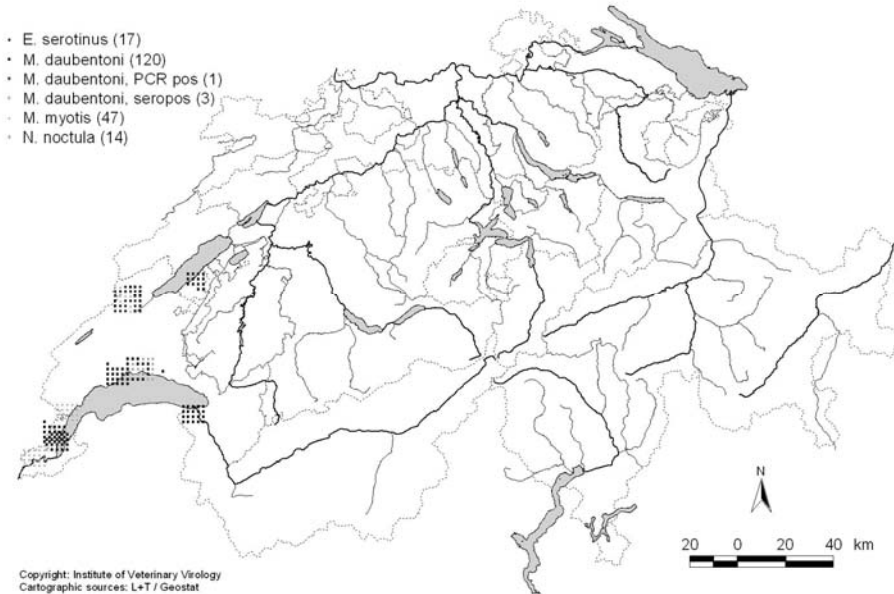


Figure 1: The distribution of records from the targeted bat rabies surveillance in Switzerland 2009. Sampling sites are indicated with coordinates of individual samples shifted for clarity. Only samples tested in both RT-PCR in saliva and in neutralization test are included. Bat species and samples with positive results are indicated.

detected in Western Europe (Müller, 1996). The present work confirms EBLV-2 infection in Daubenton's bats in Switzerland but considering the biased sampling, the epidemic picture of EBLV remains incomplete. Therefore, both active and passive surveillance must be maintained in order to assess more appropriately the potential risk for public health.

References

Müller, W.W. (1996). Review of reported rabies case data in Europe to the WHO collaborating centre Tübingen from 1977 to 1996. *Rabies Bulletin Europe* 20/4, 11-18.
Picard, E., Barrat, J., Stroucken, N., Litaize, E., Verdot, A., Patron, C., Ambert, J., Biarnais, M., & Cliquet, F. (2005). Epidémiologie des infections à lyssavirus chez les

chiroptères en France métropolitaine: bilan des analyses pour l'année 2005. *Bulletin Epidémiologique Mensuel de la Rage Animale en France* 35/10-11-12, 1-9.

Smith, J.S., Yager, P.A., & Baer, G.M. (1973). A rapid reproducible test for determining rabies neutralizing antibody. *Bulletin of the World Health Organization* 48, 535-541.

Vazquez-Moron, S., Avellon, A., & Echevarria, J.E. (2006). RT-PCR for detection of all seven genotypes of Lyssavirus genus. *Journal of Virological Methods* 135, 281-287.

Zalan, E., Wilson, C., & Pukitis, D. (1979). A microtest for the quantitation of rabies virus neutralizing antibodies. *Journal of Biological Standardization* 7, 213-220.