Tentative novel lyssavirus in a bat in Finland

T. Nokireki | N. Tammiranta | U.-M. Kokkonen | T. Kantala | T. Gadd

Finnish Food Safety Authority Evira, Virology Research Unit, Helsinki, Finland

Correspondence
T. Nokireki, Finnish Food Safety Authority Evira, Virology Research Unit, Helsinki, Finland
Email: tiina.nokireki@evira.fi

Funding information
Finnish Food Safety Authority Evira

1 | INTRODUCTION

The genus Lyssavirus includes 14 viruses accepted by the International Committee on Taxonomy of Viruses: rabies virus (RABV), Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV), European bat lyssavirus 1 (EBLV-1), European bat lyssavirus 2 (EBLV-2), Australian bat lyssavirus (ABLV), Irkut virus (IRKV), West Caucasian bat virus (WCBV), Khujand virus (KHUV), Aravan virus (ARAV), Shimonami bat virus (SHIBV), Bokeloh bat lyssavirus (BBLV) and Ikoma virus (IKOV) (ICTV, 2017). On top, three novel lyssaviruses remain as tentative species including Lleida bat lyssavirus (LLEBV), which was found in a bent-winged bat (Miniopterus schreibersii) in Spain in 2011 (Aréchiga-Ceballos et al., 2013), Gannoruwa bat lyssavirus (GBLV), which was isolated from a Greater Indian Fruit Bat (Pteropus giganteus) in Sri Lanka in 2015 (Gunawardena et al., 2016) and a lyssavirus detected in a Japanese house bat (Pipistrellus abramus) in Taiwan in 2016 (Anonymous, 2016). Lyssaviruses can be divided into phylogroups (Badrane & Tordo, 2001). Phylogroup I comprises RABV, DUVV, EBLV-1, EBLV-2, ABLV, IRKV, KHUV, ARAV, BBLV and GBLV, whereas LBV, MOKV and SHIBV form phylogroup II (Badrane & Tordo, 2001; Gunawardena et al., 2016; Kuzmin et al., 2010). WCBV, IKOV and LLEBV may be representatives of possible new phylogroups (Aréchiga-Ceballos et al., 2013; Kuzmin, Hughes, Botvinkin, Orciari, & Rupprecht, 2005; Marston et al., 2012). Bats (Chiroptera) are considered the natural reservoir for lyssaviruses; only MOKV and IKOV have never been detected in bats, and their hosts remain unknown. In humans, RABV causes about 55,000 deaths per year, mostly in Africa and Asia. Other lyssaviruses have only caused a few human deaths (Johnson et al., 2010).

In Finland, EBLV-2 has been isolated twice and antibodies have been detected in Daubenton’s bats, and it is therefore considered to be endemic in the Daubenton’s bat population (Jakava-Viljanen, Lilley, Kyheröinen, & Huovilainen, 2010; Nokireki et al., 2013). These detections have been from the western part of Finland, and the presence of lyssaviruses in other parts of the country has not been demonstrated. Thirteen species of bats have so far been recorded in Finland. The Brandt’s bat (Myotis brandtii) is one of the five common bat species in Finland and is mainly restricted to the southern part of the country. It is insectivorous and usually weighs 5–7 g. The Brandt’s bat is mainly sedentary, and the seasonal migration is usually less than 40 km. Compared to other species, it is not so often found near human settlements. During summer, it roosts in tree holes and trunk cracks, whilst, in winter, it chooses to hibernate in caves and mines. Nursery colonies usually comprise 20–60 females, and colonies mixed with Myotis mystacinus and Myotis daubentonii have been observed. In winter roosts, M. brandtii/mystacinus usually hibernate clustered up to 13 individuals. (Dietz & Kiefer, 2016; Silvennoinen & Wermundsen, 2008). It has the longest known lifespan of all bat species, exceeding 40 years (Podlutsky, Khritankov, Ovodov, & Austad, 2005).

2 | MATERIALS AND METHODS

In August 2017, a dead bat was found outside a vacation home in Eastern Finland in the municipality of Leppävirta (62°29’30”N, 027°47’15”E), in the village of Kotalahti. The bat was sent to the Finnish Food Safety Authority Evira for autopsy. The bat was a
male bat weighing 3.5 g and it was quite autolysed on arrival, with maggots inside the corpse. It was identified as a Brandt's bat by PCR method amplifying ND1 mitochondrial gene (Boston et al., 2011).

Smears prepared from brain tissue for the fluorescent antibody test (FAT) were fixed in high-grade cold acetone, air dried and then stained with specific conjugate (FITC anti-rabies monoclonal globulin, Fujirebio Diagnostics, and Rabies anti-nucleocapsid conjugate, Bio-Rad). FAT slides were examined for specific fluorescence using a fluorescence microscope.

A 10% suspension was made from the brain sample. The suspension was then inoculated in mouse neuroblastoma (MNA) cells according to a rabies tissue culture infectious test (RTCIT) procedure described in the OIE manual (OIE, 2016).

RNA was extracted from the brain suspension with the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The OneStep RT-PCR kit (Qiagen) was used to amplify two partially overlapping fragments of 612 nt and 1,026 nt of the nucleoprotein (N) gene. The reaction volume was 25 μl and the temperature profile of cDNA synthesis and amplification was 30 min at 50°C, 1 min at 95°C for reverse transcriptase inactivation and DNA polymerase activation, followed by 30 amplification cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C. The primers were published by Davis et al., (2005). The PCR products were separated on a 2% agarose gel stained with ethidium bromide. PCR products that produced bands of expected sizes on the agarose gel were purified from the gel using the Qiaquick Gel Extraction Kit (Qiagen). The PCR products were sequenced using an ABI 3100 Avant Genetic Analyzer (Applied Biosystems) with the primers used in the PCR and a Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). The sequences were analysed using DNASTAR Lasergene 10 (DNASTAR, Inc), BioEdit (http://www.mbio.ncsu.edu) and MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) software and compared with previously published lyssavirus sequences in NCBI GenBank using BLASTN program. A phylogenetic tree was constructed using maximum likelihood method, with general time-reversible correction for multiple substitutions, as implemented in MEGA software.

3 RESULTS AND DISCUSSION

The FAT slides stained with Rabies anti-nucleocapsid conjugate (Bio-Rad) showed no staining. The slides stained with FITC anti-rabies monoclonal globulin (Fujirebio Diagnostics) showed slight and atypical staining, and the possibility of lyssavirus infection could not be ruled out. In RTCIT, no viral growth was visible after four consecutive passages. In RT-PCR, the sample produced positive bands of expected sizes on the agarose gel.

N-gene sequencing produced a sequence of 1370 nt, combined from the two partially overlapping fragments amplified. Phylogenetic analysis revealed that the virus differed from other known lyssaviruses and was designated as Kotalahti bat lyssavirus (KBLV) (MF960865), a tentative new member of the genus lyssavirus (Figure 1). It shared the highest nucleotide identities of 81.0%, 79.7%, 79.5% and 79.4% with KHUV (EF614261), ARAV (EF614259), BBLV (KF245925) and EBLV-2 (EF157977), respectively.

Virus isolation was negative after four passages in MNA cell culture, perhaps because the bat was in an autolysed state or this

![FIGURE 1 Phylogenetic tree of lyssavirus sequences based on whole N-gene sequences. A phylogenetic tree constructed using maximum likelihood method, with general time-reversible correction for multiple substitutions, as implemented in MEGA software. Bootstrap values are shown next to branches. The scale bar indicates the number of nucleotide substitutions per site. Viral genome of this study (MF960865) is marked with a black square [Figure 1 replaced after initial online publication on 15 February 2018: The species name corrected from M. mystacinus to M. brandti.ii](image)
previously unknown virus might not grow in MNA cell culture as well as other bat lyssaviruses or our cell culture procedure might not be sensitive enough. The FAT-negative result with the other conjugate used raises concern over whether FAT alone or FAT together with virus isolation in cell culture is sufficient for bat samples. The sensitivities of both the FAT and RTCIT have been shown to depend on the quality of the specimen (Fooks et al., 2012; Wacharapluesadee & Hemachudha, 2010). McElhinney, Marston, Brookes, and Fooks (2014) detected viral RNA from a carcass decomposing at 25°C for 48 days, whilst virus was isolated using the RTCIT on day 3 and the FAT detected viral antigen on day 12. It is advisable that bat samples should undergo viral genome analysis when decomposed samples are likely to be submitted. As the bat was found dead, there is no information on whether it displayed clinical signs of rabies. Bats are the natural reservoir of lyssaviruses. The detection of seropositive bats without clinical signs suggests that bats can be infected and clear the infection. Lyssavirus infection can also cause clinical disease in bats. Spillover events from Chiroptera to other mammals are rare but have occurred with RABV (Daoust, Wandeler, & Casey, 1996; Leslie et al., 2006), EBLV-1 (Dacheux et al., 2009; Müller et al., 2004; Tjørnehøj, Fooks, Agerholm, & Rønsholt, 2006), MOKV (Sabeta et al., 2007), LBV (Markotter et al., 2006) and ABLV (Shinwari et al., 2007), but these infections are usually dead-end events. In addition to RABV, also ABLV, EBLV-1, EBLV-2, IRKV, DUVV and MOKV (reviewed in Johnson et al., 2010) have been responsible for human deaths, even though only in few cases. Therefore, we can assume that humans might also be susceptible to Kotalahti bat lyssavirus.

Based on the close relationship with phylogroup I viruses, vaccines available for animals and humans will probably also offer some level of cross-protection against Kotalahti bat lyssavirus, even though the sequence alone cannot provide a reliable interpretation of antigenic differences, and further studies are needed. New or tentative members of genus Lyssavirus have recently been identified from bats: Shimoni bat virus in 2009 in Kenya (Kuzmin et al., 2010), Bokeloh bat lyssavirus in 2010 in Germany (Freuling et al., 2011), Lleida bat lyssavirus in 2011 in Spain (Aréchiga-Ceballos et al., 2013), Gannoruwa bat lyssavirus in Sri Lanka in 2015 (Gunawardena et al., 2016) and bat lyssavirus in Taiwan in 2016 (Anonymous., 2016). The lyssaviruses closest to Kotalahti bat lyssavirus have been identified from Myotis spp. These are Bokeloh bat lyssavirus from Natterer’s bat (Myotis nattereri) (Freuling et al., 2011), Aravan bat virus from the lesser mouse-eared bat (Myotis blythii) (Arai, Kuzmin, Kameoka, & Botvinkin, 2003) and Khujand virus from the whiskered bat (M. mystacinus) (Kuzmin, Botvinkin, & Khabilov, 2001). According to our knowledge, no previous detections of lyssavirus have been made in Brandt’s bat. Whether the Brandt’s bat is the reservoir species for Kotalahti bat lyssavirus remains unclear and requires further investigation. Brandt’s bat species have been included in passive surveillance in Finland but only in low numbers, 11 Brandt’s bats were tested during 1986–2016. Six Brandt’s bats were analysed in Sweden in passive surveillance during 1991–2011 (reviewed by Schatz et al., 2013). In Germany, altogether, 59 Brandt’s bats were analysed for rabies 1998–2014, all with negative results (Schatz et al., 2014). Many bats have not been identified to species level, but, nevertheless, it seems that low numbers of Brandt’s bat have been tested for the presence of lyssaviruses in Europe.

ACKNOWLEDGEMENTS

We thank Marja Isohelsinki, Riikka Holopainen and Tiina Peltonen for their contribution. This work was funded by Finnish Food Safety Authority Evira.

CONFLICT OF INTEREST

None declared.

ORCID

T. Nokireki http://orcid.org/0000-0003-2407-9262

REFERENCES

Anonymous. (2016, November 14). Novel bat lyssavirus - Taiwan. (Tainan). Retrieved from http://www.promedmail.org/post/20161114.4627752

Arai, Y. T., Kuzmin, I. V., Kameoka, Y., & Botvinkin, A. D. (2003). New lyssavirus genotype from the lesser mouse-eared bat (Myotis blythii) Kyrgyzstan. Emerging Infectious Diseases, 9(3), 333–337. https://doi.org/10.3201/eid0903.020252

Aréchiga-Ceballos, N. A., Morón, S. V., Berçiano, J. M., Nicolás, O., López, C. A., Juste, J., … Echevarría, J. E. (2013). Novel lyssavirus in bat Spain. Emerging Infectious Diseases, 19(5), 793–795. https://doi.org/10.3201/eid1905.121071

Badrane, H., & Tordo, N. (2001). Host switching in Lyssavirus history from the Chiroptera to the Carnivora orders. Journal of Virology, 75 (17), 8096–8104. https://doi.org/10.1128/JVI.75.17.8096-8104.2001

Boston, E. S. M., Hanrahan, N., Puechmaille, S. J., Ruedi, M., Buckley, D. J., Lundy, M. G., & Teeling, E. C. (2011). A rapid PCR-based assay for identification of cryptic Myotis spp. (M. mystacinus, M. brandti and M. alcathe). Conservation Genetics Resources, 3(3), 557–563. https://doi.org/10.1007/s12686-011-9404-9

Dacheux, L., Larrous, F., Mailles, A., Boisserieau, D., Delmas, O., Biron, C., … Bourhy, H. (2009). European bat lyssavirus transmission among cats Europe. Emerging Infectious Diseases, 15(2), 280–284. https://doi.org/10.3201/eid1502.080637

Daoust, P. Y., Wandeler, A. L., & Casey, G. A. (1996). Cluster of rabies cases of probable bat origin among red foxes in Prince Edward Island Canada. Journal of Wildlife Diseases, 32(2), 403–406. https://doi.org/10.7589/0090-3558-32.2.403

Davis, P. L., Holmes, E. C., Larrous, F., Van der Poel, W. H., Tjørnehøj, K., Alonso, W. J., & Bourhy, H. (2005). Phylogeography, population dynamics and molecular evolution of European bat lyssaviruses. Journal of Virology, 79(16), 10487–10497. https://doi.org/10.1128/JVI.79.16.10487-10497.2005

Dietz, C., & Kiefer, A. (2016). Brandt’s Bat. In Bats of Britain and Europe. London: Bloomsbury Publishing.

Fooks, A. R., McElhinney, L. M., Horton, D., Banyard, A., Johnson, N., Marston, D. A., … Rupprecht, C. E. (2012). Molecular tools for rabies diagnosis in animals. In A. R. Fooks & T. Müller (Eds.), Compendium of...
the OIE Global Conference on Rabies Control (pp. 75–87). Paris: World Organisation for Animal Health.

Freuling, C. M., Beer, M., Conraths, F. J., Finke, S., Hoffmann, B., Keller, B., & Müller, T. (2011). Novel lyssavirus in Natterer’s bat Germany. Emerging Infectious Diseases, 17(8), 1519–1522. https://doi.org/10.3201/eid1708.110201

Gunawardena, P. S., Marston, D. A., Ellis, R. J., Wise, E. L., Karawita, A. C., Breed, A. C., … Fooks, A. R. (2016). Lyssavirus in Indian flying foxes Sri Lanka. Emerging Infectious Diseases, 22(8), 1456–1459. https://doi.org/10.3201/eid2208.151986

International Committee on Taxonomy of Viruses. (2017, November 16). Retrieved from https://talk.ictvonline.org/taxonomy/

Jakava-Viljanen, M., Lilley, T., Kyheroinen, E.-M., & Huovilainen, A. (2010). First encounter of European bat lyssavirus type 2 (EBLV-2) in a bat in Finland. Epidemiology & Infection, 138(11), 1581–1585. https://doi.org/10.1017/S095026881000373

Johnson, N., Vos, A., Freuling, C., Tordo, N., Fooks, A. R., & Müller, T. (2010). Human rabies due to lyssavirus infection of bat origin. Veterinary Microbiology, 142(3–4), 151–159. https://doi.org/10.1016/j.vetmic.2010.02.001

Kuzmin, I. V., Botvinkin, A. D., & Khabilov, T. K. (2001). The lyssavirus was isolated from a whiskered bat in northern Tajikistan. Plecotus et al, 4, 75–81.

Kuzmin, I. V., Hughes, G. J., Botvinkin, A. D., Orciari, L. A., & Rupprecht, C. E. (2005). Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. Virus Research, 111(1), 28–43. https://doi.org/10.1016/j.virusres.2005.03.008

Kuzmin, I. V., Mayer, A. E., Niezgoda, M., Markotter, W., Agwanda, B., Breiman, R. F., & Rupprecht, C. E. (2010). Shimoni bat virus, a new representative of the Lyssavirus genus. Virus Research, 149(2), 197–210. https://doi.org/10.1016/j.virusres.2010.01.018

Leslie, M. J., Messenger, S., Rohde, R. E., Smith, J., Cheshier, R., Hanlon, C., & Rupprecht, C. E. (2006). Bat-associated rabies virus in skunks. Emerging Infectious Diseases, 12(8), 1274–1277. https://doi.org/10.3201/eid1208.051526

Markotter, W., Randles, J., Rupprecht, C. E., Sabeta, C. T., Taylor, P. J., Wandelers, A. I., & Nel, L. H. (2006). Lagos bat virus South Africa. Emerging Infectious Diseases, 12(3), 504–506. https://doi.org/10.3201/eid1203.051306

Marston, D. A., Horton, D. L., Ngeleja, C., Hampson, K., McElhinney, L. M., Banyard, A. C., … Lembo, T. (2012). Ikoma lyssavirus, highly divergent novel lyssavirus in an African civet. Emerging Infectious Diseases, 18(4), 664–667. https://doi.org/10.3201/eid1804.111553

McElhinney, L. M., Marston D. A., Brooks, S. M., & Fooks, A. R. (2014). Effects of carcass decomposition on rabies virus infectivity and detection. Journal of Virological Methods, 207, 110–113. https://doi.org/10.1016/j.jviromet.2014.06.024

Müller, T., Cox, J., Schafer, R., Johnson, N., McElhinney, L. M., Geue, J. L., … Fooks, A. R. (2004). Spill-over of European bat lyssavirus type 1 into a stone marten (Martes foina) in Germany. Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health, 51(2), 49–54. https://doi.org/10.1111/j.1439-0450.2003.00725.x

Nokireki, T., Huovilainen, A., Lilley, T., Kyheroinen, E.-M., Ek-Kommonen, C., Sihvonen, L., & Jakava-Viljanen, M. (2013). Bat rabies surveillance in Finland. BMC Veterinary Research, 9, 174. https://doi.org/10.1186/1746-6148-9-174

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (2016, November 16). Chapter 2.1.17. Rabies (infection with rabies virus). Retrieved from http://www.oie.int/fileadmin/Home/eng/Heath_standards/tahm/2.01.17_RABIES.pdf

Podulsky, A. J., Khritankov, A. M., Ovodov, N. D., & Austad, S. N. (2005). A new field record for bat longevity. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 60(11), 1366–1368. https://doi.org/10.1093/gerona/60.11.1366

Sabela, C. T., Markotter, W., Mohale, D. K., Shumba, W., Wandelers, A. I., & Nel, L. H. (2007). Mokola virus in domestic mammals South Africa. Emerging Infectious Diseases, 13(9), 1371–1373. https://doi.org/10.3201/eid1309.070466

Schatz, J., Fooks, A. R., McElhinney, L., Horton, D., Echevarria, J., Vázquez-Moron, S., … Freuling, C. M. (2013). Bat rabies surveillance in Europe. Zoonoses Public Health, 60(1), 22–34. https://doi.org/10.1111/zph.12002

Schatz, J., Freuling, C. M., Auer, E., Goharriz, H., Harbusch, C., Johnson, N., … Müller, T. (2014). Enhanced passive bat rabies surveillance in indigenous bat species from Germany – A retrospective study. PLoS Neglected Tropical Diseases, 8(5), e2835. https://doi.org/10.1371/journal.pntd.0002835

Shinwari, M. W., Annand, E. J., Driver, L., Warrillow, D., Harrower, B., Allcock, R. J., … Diolo, L. S. (2014). Australian bat lyssavirus infection in two horses. Veterinary Microbiology, 173(3–4), 224–231. https://doi.org/10.1016/j.vetmic.2014.07.029

Siivonen, Y., & Wermundsen, T. (2008). Characteristics of winter roosts of bat species in southern Finland. Mammalia, 72(1), 50–56. https://doi.org/10.1515/mamm.2008.003

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30(12), 2725–2729. https://doi.org/10.1093/molbev/msr197

Tjarnehøj, K., Fooks, A. R., Agerholm, J. S., & Rensholt, L. (2006). Natural and experimental infection of sheep with European bat lyssavirus type-1 of Danish bat origin. Journal of Comparative Pathology, 134(2–3), 190–201. https://doi.org/10.1016/j.jcpa.2005.10.005

Wacharapluesadee, S., & Hemachudha, T. (2010). Ante- and post-mortem diagnosis of rabies using nucleic acid-amplification tests. Expert Review of Molecular Diagnostics, 10(2), 207–218. https://doi.org/10.1586/erm.09.85

---

How to cite this article: Nokireki T, Tammiranta N, Kokkonen U-M, Kantala T, Gadd T. Tentative novel lyssavirus in a bat in Finland. Transbound Emerg Dis. 2018;00:1–4. https://doi.org/10.1111/tbed.12833