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Short communication

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Detection of European bat lyssavirus 2 (EBLV-2) in a Daubenton's bat (*Myotis daubentonii*) from Magdeburg, Germany

Nachweis von Fledermaustollwut (EBLV-2) bei einer Wasserfledermaus (Myotis daubentonii) aus Magdeburg

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Summary

In Europe bat rabies in Daubenton's bats (*Myotis daubentonii*) and in Pond bats (*Myotis dasycneme*) caused by the European bat lyssavirus 2 (EBLV-2) has been confirmed in less than 20 cases to date. Here we report the second encounter of this virus species in Germany. A Daubenton's bat found grounded in the zoological garden in Magdeburg died shortly after. In the frame of a retrospective study the bat carcass was eventually transferred to the national reference laboratory for rabies at the Friedrich-Loeffler-Institute for rabies diagnosis. Lyssavirus was isolated and characterized as EBLV-2.

Keywords: bat rabies, lyssavirus, EBLV-2, *Myotis daubentonii*

Zusammenfassung

In Europa ist die Fledermaustollwut bei Wasserfledermäusen (*Myotis daubentonii*) und Teichfledermäusen (*Myotis dasycneme*), die durch das Europäische Fledermaustollwutvirus Typ 2 (EBLV-2) hervorgerufen wird, bisher nur in weniger als 20 Tieren nachgewiesen worden. Hier wird über einen weiteren Nachweis von EBLV-2 bei einer Wasserfledermaus, die im Zoo Magdeburg aufgefunden wurde, berichtet. Das Tier war flugunfähig aufgefunden worden und verstarb kurze Zeit später. Im Rahmen einer retrospektiven Studie gelangte es zur Tollwutuntersuchung und das isolierte Virus wurde als EBLV-2 charakterisiert.

Schlüsselwörter: Fledermaustollwut, Lyssavirus, EBLV-2, *Myotis daubentonii*

Introduction

Only after the tragic death of a Swiss biologist in Finland in 1985 and subsequent characterization of the causative agent it became evident that bat rabies in Europe at that time was caused by two different lyssavirus species: European bat lyssavirus 1 and 2 (EBLV-1 and 2) (Bourhy et al., 1992; Lumio et al., 1986). In the meantime West Caucasian bat lyssavirus (WCBV) was isolated from a bat at the southeast border of Europe (Kuzmin et al., 2005), and recently Bokeloh bat lyssavirus (BBLV) was found in a Natterer's bat (*Myotis nattereri)* from Germany (Freuling et al., 2011).

Of more than 900 reported bat rabies cases in Europe from 1977 to 2010 (Rabies Bulletin Europe) less than 20 have been identified as EBLV-2 (Tab. 1), including a virus isolate from a second human rabies case in the UK (Fooks et al., 2003). Besides the UK with the most confirmed cases, EBLV-2 has also been isolated from bats in Switzerland, Finland, and The Netherlands. Except for The Netherlands where Pond bats (*Myotis dasycneme*) were found to be infected, all EBLV-2 were isolated from Daubenton's bats (*Myotis daubentonii*) (Tab. 1).

In Germany, the first evidence for the circulation of EBLV-2 was found in 2007, when a Daubenton's bat from Bad Buchau, Baden-Wuerttemberg tested positive. This grounded bat was taken to a local bat sanctuary, but was subsequently euthanized as a result of its bad condition (Freuling et al., 2008). Here we report the second isolation of EBLV-2 from a Daubenton's bat from Germany.

The case

On September 21, 2006, an adult male Daubenton's bat was found by a member of the public at the Zoological garden of Magdeburg (Fig. 1). It was taken to the zoo's rescue centre for rehabilitation without further contacts to other people. In captivity, the bat was recorded as being unable to fly, dehydrated and with 6 g of body weight being below the range of 7–15 g (Schober and Grimmberger, 1987). The bat was reluctant to take fluids or feed and even manually applied water using a syringe was not swallowed and caused choking. One day later the bat succumbed and its carcass was stored in a deep freezer in compliance with the regulation for the preservation of bats in Saxony-Anhalt. Subsequently the bat was submitted in the framework of passive surveillance for bat lyssaviruses to the national reference laboratory (NRL) for rabies testing.

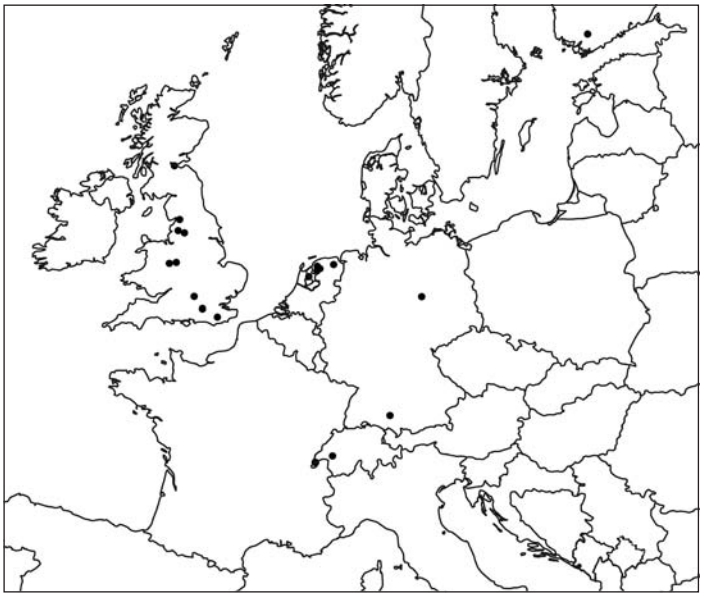

FIGURE 1: *Distribution of documented EBLV-2 cases in bats in Europe.*

In September 2008, lyssavirus antigen was detected by the standard rabies fluorescent antibody test (FAT; Dean et al., 1996) in the brain. During subsequent testing virus was isolated in cell culture using the rabies tissue infection test (RTCIT; Webster and Casey, 1996) and EBLV-2 specific RNA was detected with an RT-PCR amplification assay in original brain material (Vos et al., 2004). A modified semi-nested RT-PCR (Heaton et al., 1997), targeting the nucleoprotein (N) gene was used to generate a PCR fragment for subsequent sequencing as described before (Freuling et al., 2008). Sequence analysis of the first 400 bp of the N-gene showed 98.7% identity to another German EBLV-2 isolate from a Daubenton's bat in Bad Buchau in 2007. The close genetic association is shown in Figure 2. Virus specific nucleic acid was also detected by an in-house realtime RT-PCR in most peripheral organ tissues investigated (Tab. 2).

TABLE 1: *Documented cases of EBLV-2 in Myotis bats in Europe*

Year	*Myotis* species	Country	Origin	Reference
1987	*Myotis dasycneme*	The Netherlands	Wommels	Davis et al., 2005
			Tjerkwerd	Nieuwenhuijs et al., 1992
			Tjerkgaast	
1989	*Myotis dasycneme*	The Netherlands	Andijk	Davis et al., 2005
1992	*Myotis daubentonii*	Switzerland	Plaffeien	Megali et al., 2010
1993			Versoix	
1993	*Myotis dasycneme*	The Netherlands	Roden	Davis et al., 2005
1996	*Myotis daubentonii*	United Kingdom	New Haven, Sussex	Banyard et al., 2009
2002			Carnforth, Lancashire	
2003			Staines, Surrey	
2004			Blackburn, Lancashire	
2006		Germany	Magdeburg	This article
2006		United Kingdom	Abingdon,Oxfordshire	Banyard et al., 2009
2007		Germany	Bad Buchau	Freuling et al., 2008
2007		United Kingdom	Stokesay Castle	Banyard et al., 2009
2008			Teddington, Surrey	
2009			Stokesay Castle	
2009			Linlithgow, West Lothian	Horton et al., 2009
2009		Finland	Turku	Jakava-Viljanen et al., 2010

Discussion

This second EBLV-2 case is the first found in the lowlands of Germany indicating that bat rabies caused by EBLV-2 is more widely distributed in Europe and Germany than reported previously (Tab. 1). As in the previous case the bat species involved was a Daubenton's bat. Even though being stored in a freezer for more than a year viral RNA was found in the brain and in several peripheral tissues (Tab. 2). The highest viral load was observed in brain material, supporting the hypothesis of infection of peripheral organs through centrifugal

dissemination from the brain. The presence of viral RNA in peripheral organs is likely a result of innervations, and the heterogenic pattern in different non-neuronal organs observed in experimental settings (Johnson et al., 2008) as well as other field data (Banyard et al., 2009; Freuling et al., 2008; Johnson et al., 2003) may be linked to the relative time point of death and the degree of centrifugal viral dissemination. Given the comparatively lower amount of tissue from the salivary gland used for RNA-extraction and subsequent amplification, the detection of higher levels of virus RNA in this organ supports the general understanding that virus is transmitted in the saliva via shallow bites (Johnson et al., 2008).

Two bat handlers became victim of bat transmitted EBLV-2 infections, underscoring the public health risk of this bat rabies variant. Also in Germany, both cases of EBLV-2 in Daubenton's bats involved people that had direct or indirect contact with the bat. While in the first documented case, the person that was bitten had just completed their prophylactic vaccination (Freuling et al., 2008), there was no indication that the bat in this case, in the zoo of Magdeburg, had bitten any person involved. However, grounded bats with unusual behavior seem predestined for being tested rabies positive as also observed in the UK (Banyard et al., 2009). Thus, this case supports the need for bat handlers and especially rehabilitators to be vaccinated against rabies as also required by international and national guidelines (Delere et al., 2011; World Health Organisation, 2005). Also, this case underscores the need for continual education of the public in the risks of rabies transmitted by bats and the importance of passive surveillance schemes for bat rabies. EUROBATS encourages a bat rabies surveillance system in all countries (Anon, 2006), and such a passive surveillance scheme for bat rabies was initiated by the NRL in the year 2000 (unpublished). Because of Germany's federalism different approaches for the preservation of dead found bats exist among the federal states which make it difficult to have a common approach. In Saxony-Anhalt, deceased bats have to be stored in a freezer and are eventually transferred to the Museum of Natural History at the University of Halle. Due to cooperation with the NRL for Rabies at the FLI in the frame of a retrospective study (passive surveillance) following morphometric analysis and speciation

TABLE 2: *Organ distribution of viral RNA using a realtime RT-PCR*

Organ tissue	Presence of viral RNA	Cycle threshold
Brain	+	25.14
Tongue	+	34.47
Heart	+	32.86
Lung	+	34.47
Pectoral muscle	+	38.32
Liver	+	39.21
Kidney	+	38.90
Spleen	-	-
Bladder	-	-
Neg control	-	-
Pos control (10^{-7})	+	35.89

bat carcasses are then routinely submitted for rabies diagnosis. From more than 2000 bats tested in the frame of rabies routine testing in regional veterinary laboratories in Germany before, bats from various locations in northern Germany all tested EBLV-1 positive (Müller et al., 2007). This is the first EBLV-2 case in the frame of passive surveillance in Germany. However, as Daubenton's bats are underrepresented in passive surveillance (unpublished) there is reason to believe that more cases were detected if the number of submissions would increase. Because of the pathogenesis of rabies with animals showing clinical symptoms and eventually succumb to the disease, the highest chance to find the virus is in moribund or deceased animals. If stored at –20°C it is possible to confirm the diagnosis even years after death as was also reported in a case from the UK (Fooks et al., 2004).

Although it is the second reported case of EBLV-2 in Germany, according to the time of death of the bat, however, it represents the earliest verification of EBLV-2 in Germany and is at the same time the earliest report in the context of passive surveillance.

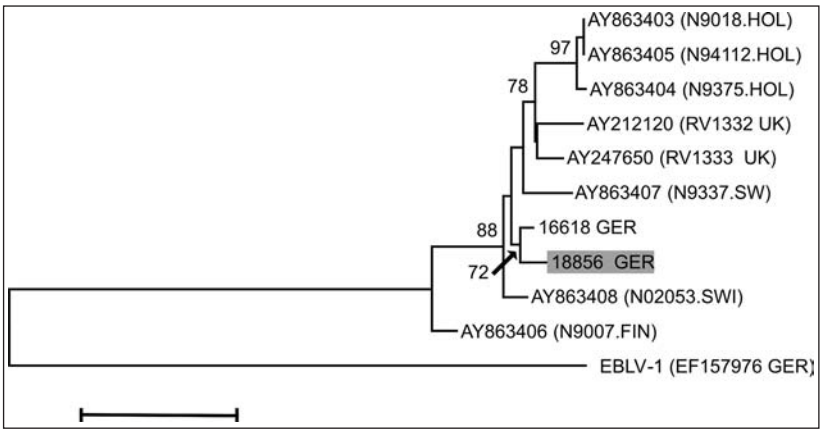

FIGURE 2: *Phylogenetic tree using a fragment of the N-gene (position 1-400) from EBLV-2 isolates in Europe with EBLV-1 (EF 157976 GER) as outgroup. The new isolate 18856 GER is highlighted. The neighbour-joining method (Kimura 2-parameter) as implemented in MEGA5 was applied with bootstrap values (1000 replicates) shown next to branches.*

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This manuscript is dedicated to Dr. Dietrich Heidecke who suddenly died during the publication process. Dr. Heidecke acted as custodian and was responsible for the vertebra at the Zoological Collections of the Martin-Luther-University.

Conflict of interest: The authors declare that no competing interests exist.

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