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International Research Grant report

The European bat lyssaviruses (EBLVs) are a viral group closely related to the rabies virus and are capable of causing a rabies-like disease in both animals and man. The UK has recorded five cases, all of the type 2 (EBLV-2) subgroup, of which four were isolated from Daubenton's bats (*Myotis daubentonii*). In contrast, Germany has recorded 70 cases since 1997, all of type 1 (EBLV-1), mainly in the serotine bat (*Eptesicus serotinus*). Diagnosis of rabies and rabies-like disease in Germany is the responsibility of the 16 federal states. In addition to this, the German government funds a reference laboratory that provides a diagnostic service for rabies, co-ordinates anti-rabies vaccination campaigns (against fox rabies) and archives submitted virus isolates. This service is based at the Friedrich-Loeffler-Institute (FLI), Wusterhausen, approximately 60 miles north of Berlin. I visited the institute with the aim of gaining access to the EBLV-1 isolate archive and carrying out epidemiological studies to compare these isolates to previous German

EBLV-1 isolates and to those from throughout Europe.

The first reports of a rabies virus associated with bats were recorded in Germany, mainly in German-language journals. A further benefit of visiting Germany has been to obtain these papers and gain assistance in translating them. These early reports, from the 1950s, described rabies-like disease in bats and suggested an early link with the serotine species. The first study describing the epidemiology of rabies viruses in bats was that of Siedler *et al.* (1987). The authors reported the distribution of cases in Lower Saxony, a federal state bordering The Netherlands, another country that has reported numerous cases of rabies in bats. Since this time, the rabies-like viruses infecting bats in Europe have been shown to be distinct from classical rabies and named European bat lyssaviruses. German EBLV-1 isolates have been included within larger molecular epidemiological studies on EBLVs and have been shown to be a closely related group. However, a detailed investigation



Molecular epidemiology of *European bat lyssavirus 1* based on comparison of the viral nucleoprotein

▲ Serotine bat in flight. © Kim Taylor / naturepl.com

of EBLV-1 isolates from Germany, linking phylogenetic variation with geographical distribution, has not been conducted. Thirty-six EBLV-1 viruses were included in this study from the Wusterhausen archive, all submitted since 1997. Viral RNA was extracted and reverse-transcribed using a pan-lyssavirus primer. Fragments of the viral nucleoprotein gene and the nucleoprotein/phosphoprotein intergenic junction (N-P) were PCR-amplified. Whilst in Wusterhausen, N-P sequences were obtained and compared to further isolates from Europe (including sequences from Denmark, Poland, The Netherlands, France and Spain). A preliminary phylogenetic tree suggests a clustering of viral sequences dependent on geographical location. The sample group is dominated by sequences from northern Germany. This could be a result of biased sampling or a focus of EBLV-1 infection. EBLV-1 isolates have been reported in north-east Germany since the 1960s, so it would be fair to suspect that the virus has persisted in this region over four decades. The highest numbers of serotine bats are estimated to be in the federal states of Lower Saxony, Schleswig-Holstein and Mecklenburg-Western Pomerania, the three most northerly federal states of Germany (Bats and Bat Conservation in Germany: www.bfn.de). This may enable EBLV-1 to persist in these areas in a manner similar to classical rabies where numbers of susceptible hosts are critical to the survival of the virus. This group of EBLV-1 is closely related to those from The Netherlands and Denmark.

Virus sequences from other areas of Germany, Saarbrücken to the south and Stendal in central Germany, show closer links to viruses from France and Poland, respectively. Again, this must be linked to the behaviour of the host, which is non-migratory and thus does not allow widespread dissemination of particular lineages of EBLV-1.

It is clear that further work is required on this panel of samples and the cDNAs for all isolates studied have now been sent to the UK. This should allow a better separation of isolates using a longer nucleoprotein sequence of the EBLV genome for phylogenetic analysis. In addition, studies on the behaviour of the host, in Germany the serotine bat and the Daubenton's bat in the UK, may be as instructive in explaining the distribution and persistence of the European bat lyssaviruses than investigation of the virus alone.

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