

Final report - Importance of chiropteran-borne viruses: detection, isolation and molecular characterization of potentially emerging novel zoonotic viruses (KH_129599)

In accordance with the grant proposal we performed viral metagenomic screening of bat samples. As a significant result of these experiments we discovered a novel hantavirus in a fairly investigated bat species. We used the Oxford Nanopore, third-generation sequencing technology for viral metagenomic experiments. During the time of the study this methodological effort was a leading step towards the integration of Nanopore technology into the NGS-based viral discovery efforts. We obtained genomic information of all three genomic segments of the virus, which permitted the detailed phylogenetic evaluation and taxonomic positioning. The novel virus was tentatively named as Sarawak mobatvirus (SARV) within the Mobatvirus genus and it was detected in the Bronze tube-nosed bats (*Murina aenea*, Vespertilionidae). This bat species is a member of *Murina* genus, which is a special bat group regarding its ecological needs, lifestyle and behaviour. Hantaviruses are among one of the most important rodent-borne zoonotic viruses worldwide, with a huge undiscovered gene-pool in other animals, mostly in bats. We published the novel results and described this novel virus with discussion on its potential zoonotic nature in *Viruses* journal (MDPI) (Zana et al., 2019). This is a significant step forward to better understand the evolution of these viruses and also a significant added value to the ongoing risk assessment of novel, possibly zoonotic viruses discovered worldwide.

In addition to viral discovery we performed extensive evolutionary analysis on a novel picornavirus, described in our laboratory. This work was a step forward in our previous research activities in Hungary, when we described a novel member of the Mischivirus genus within the viral family of Picornaviruses in Schreiber's bats. We extended the geographical scope of this project and examined Schreiber's bat samples from Algeria. As a result of this investigation we revealed the presence of the same virus within these distant bat populations, which opened the possibility to analyse evolutionary and transmission patterns between bat hosts. We used state-of-the-art analytic tools and powerful phylogenetic analyses to examine host switch events during the evolution of bat-related picornaviruses. We published the fundamentals of the evolution of the family Picornaviridae in bat hosts and compared the novel genomic sequence data with all bat-related picornaviruses. This extensive viral discovery and evolutionary analysis work was published in *Scientific Reports* journal. The results we presented in this manuscript help to understand the basic evolutionary mechanisms of these viruses within their hosts (host jump events, recombination, etc) and finally help to better understand the spillover

risk and zoonotic potential for humans. The work is unique in the field of bat virology and presents the only insight into the evolution of bat-related picornaviruses to date.

During the grant period we investigated different levels and therefore targeted different virus groups to examine different aspects of zoonotic spillover events from bats to humans. Within the framework of our activities the examination of filoviruses in bats represented a priority objective.

Apart from viral discovery work we performed a temporal surveillance of the only known filovirus from Europe, the Lloviu cuevavirus. We continuously monitored the virus in a single *Miniopterus schreibersii* colony, Hungary with active surveillance methods. During these field activities we used our innovative field-laboratory capability and performed viral diagnostics and complete viral genomic sequencing on-site. This is a unique approach which permitted the fast identification of actively infected bats and opened the possibility to re-sample these individuals. Basic attributes of the natural circulation, novel sequence data and most importantly, the successful isolation of the virus was achieved during this work. It makes this virus only the second filovirus ever isolated from bats, following Marburg virus isolation from *Rousettus aegyptiacus* bats. In relation to this result we were able to establish novel research collaborations with researchers from multiple countries (Italy, USA, Japan, United Kingdom). The manuscript in relation to this work was recently accepted for publication in *Nature Communications* journal. It is also available as a preprint in BioRxiv server. This is a significant result in the field of virology and opened the possibility to better understand these highly pathogenic viruses with zoonotic origin. This virus is the only member of Filoviridae family ever isolated apart from the Ebola and Marburg genera. In addition to human health relevance, this result an additional significant step forward to include bats as the natural hosts or reservoirs for filoviruses with human health relevance. We were able to test the possibility of human infection in vitro within our high-containment laboratory. We demonstrated the susceptibility of multiple human cell lines and animal cell lines for Lloviu virus infection, raising the possibility for human infections in Europe.

Summary of these results:

- Discovery and genetic evaluation of a novel member within the Hantaviridae family
- Extended evolutionary analysis of bat-harboured picornaviruses and the discovery of a novel strain
- Isolation of a filovirus from bats in Europe and in vitro confirmation of human pathogenic potential