Final report of FK131465 grant - Epizootiological investigation and conservation biological effects of bat-harboured viruses

General summary:
The actuality and importance of the research topic is well indicated by the growing scientific interest in bats as one of the most important virus reservoirs. The actuality of the project is also well highlighted by the COVID-19 pandemic, which was started during the current proposal and further highlights the importance of bats as pathogen reservoirs. Although the pandemic situation affected the actual activities of the proposal and some of these were postponed or cancelled (detailed in the last paragraph), we were able to reach significant scientific output with notable international highlights of the current work. These results are strongly in accordance with the initial research goals since we were able to identify, characterize and isolate a bat-related filovirus in Europe in a highly protected bat species with presumable connection to its late-winter die-off events. Also, we identified a filovirus for the first time in non-vertebrate organisms. Overall, six conference presentations (with three invited speech) and four publications were achieved as the output of this project.

Filoviruses are among the most significant zoonotic viruses with high mortality and morbidity. While ebolaviruses have received significant attention from the public and health experts due to past outbreaks, including the West African Ebola outbreak in 2013–2016 and a recent outbreak in Uganda, other filoviruses also pose a potential threat to human and animal health. Whilst the natural reservoir for Marburg virus, the Rousettus aegyptiacus fruit bat is well characterized, the natural host of other filoviruses are still uncertain.

We performed multiple sampling occasions at the greatest known *Miniopterus schreibersii* bat colony in Hungary and other European countries. During these field trips we collected blood, urine, faeces and ectoparasite samples. A notable result of our work is the establishment and optimalization of on-site surveillance methods for the rapid and effective detection of specified pathogens. Based on this capacity we were able to re-sample bat individuals with verified and active virus infection. This approach helped the success in understanding the role of these bats as the natural host for Lloviu virus and to obtain the second in the world filovirus isolate directly from a bat. Our results are also special, since this is the first filovirus isolated from temperate climate, therefore it opened a range of possibilities in understanding these viruses in general and to better prepare for current and future outbreaks.

Summary and relevance of publications in relation to the project proposal:

- Until the availability of plain sequence data, we performed a collaborative work with partners at the National Emerging Infectious Diseases Laboratories, Boston University, USA to better understand the zoonotic nature of Lloviu virus: Next-generation sequencing has uncovered numerous RNA viruses in animal hosts, some closely related to human pathogens, but many remain unexplored due to challenges in culturing them. ***The study successfully used minigenome systems to fill in missing genomic sequences of Lloviu virus (LLOV), an uncultured filovirus related to Ebola, enabling the creation of infectious recombinant LLOV. This recombinant virus, which can infect cells targeted by Ebola and does not induce typical inflammatory responses, offers a new avenue for testing antivirals and enhances pandemic preparedness.*** This work was published in Plos Pathogens Journal.

- During this work we continued the sampling activities and the in vitro work. As a major breakthrough in the project and also in the whole field of filovirus virology, we were able to isolate the wild-type virus in vitro. Also, ***we described the basic ecology and transmission patterns of the virus*** in collaboration with several foreign colleagues from the USA, UK and Belgium: Multi-year surveillance in Hungary shows LLOV presence in both living and deceased bats and their ectoparasites, suggesting bats, especially Miniopterus schreibersii, as hosts for LLOV in Europe and implicating bat-associated parasites in the ecology of filoviruses in temperate climates. ***We were able to isolate the wild-type virus from the blood sample of bats and presented its ability to infect human, monkey, and bat cell lines. These results are in accordance with the zoonotic potential of this virus.*** This work was published in Nature Communications journal and led to three conference invitations during the grant period and additional after.

- To highlight the importance and impact of our results for outbreak prevention and conservation of these bats we published a manuscript to discuss these issues and give guidelines for other researchers and professionals working with these animals. We published this work in Ecohealth Journal: Emerging infectious diseases are significantly impacting animal populations. Lloviu filovirus (LLOV), which has caused mass mortalities in European bat populations, particularly Schreiber's bats is a growing concern to conservation biology and human spillover infections. LLOV, a member of the Filoviridae family, has demonstrated the ability to infect monkey and human cells, supporting its zoonotic potential. Strategies to mitigate its impact include molecular and serological studies of bats and their environments, population genetic studies, strict control measures in bat habitats, and regular monitoring of bat colonies, emphasizing the need for international collaboration and research to address these emerging threats. In this manuscript ***we provided point-by-point recommendations for professionals to mitigate these risks***.

- We expanded the study focus to other regions in Europe to better understand the large-scale impact of the virus. We published the first results in Scientific Reports journal: Recent studies have confirmed the zoonotic potential of both recombinant and wild-type LLOV isolates in vitro. This study ***expanded the known distribution of LLOV*** by detecting and sequencing a positive sample from Italy in 2020 and creating an infectious virus isolate. The successful establishment of this isolate using the SuBK12-08 cell line confirms the susceptibility of these cells to LLOV infection and reinforces the ***role of bats as natural hosts for zoonotic filoviruses***.

Impact of research:

Although our research is still ongoing, our findings have led other researchers to suspect the Lloviu virus as a cause behind the early spring die-offs of bent-winged bats and gave the technical and methodological toolkit to perform specific and sensitive diagnostics. Since we provided the first filovirus isolate outside the ebola and marburg genera, a number of research activities sparked out from our isolate. To better understand the human pathogenicity of the virus, in collaboration with leading filovirus research labs in the USA we already published a collaborative manuscript in The Journal of Infectious Diseases (Fletcher P, et al. Pathogenicity of Lloviu and Bombali Viruses in Type I Interferon Receptor Knockout Mice. J Infect Dis. 2023 Nov 13;228(Supplement_7):S548-S553. doi: 10.1093/infdis/jiad226.).

We provided the first insights into the ecology and transmission of a filovirus in temperate climate and revealed the bat flies and ticks as possible transmission vectors. By providing the first in vitro isolates we opened a wide range of research possibilities.

In collaboration with our partners, we contributed to the development and experimental validation of a recombinant Lloviu virus strain, marking a significant advancement in virological techniques in Hungary. This collaborative effort paves the way for comprehensive characterization of potentially hazardous pathogens that, until now, have been identified only through their genetic sequences.

Mentoring:

- One BSc student defended her thesis in the topic of Lloviu research.
- Within the framework of the project, one MSc student won the first prize at the National Scientific Students' Associations Conference (OTDK) – Biology Section 2023.
- She also won the New National Excellence Programme for MSc students in 2023 with this work.
- One PhD student reached the pre-degree certificate and started to prepare his PhD thesis in relation to this project.
- As an honour for our achievements in filovirus research, the National Biomedical Foundation awarded us the Szent-Györgyi Talent Award in 2023.
- The University of Pécs awarded the Mihály Pekár Award for the best scientific paper in 2023 at the university to the Nature Communications Lloviu paper.
- The project's Principal Investigator (PI) was awarded the Bolyai Scholarship (2019) and successfully completed it with the highest evaluation at its conclusion.
- The project's Principal Investigator (PI) was repeatedly awarded the Bolyai Scholarship (2023)

Networking

We reached several valuable networking achievements and built strong collaborative partnership with several European and multiple research institutes from the USA. As a result, and during the grant period, we earned a R21 NIH grant for bat immunology research. We submitted multiple grants with these partners, such as R01 NIH and Horizon grants.

The PI of the project was invited to three international conferences during the project (10th International Filovirus Symposium, San Diego USA, September 18-21, 2022; 3rd International Symposium on Infectious Diseases of Bats, Fort Collins USA, July 24-27, 2022; Emerging Viruses

| 2023 Oxford University, St. Edmund Hall, UK, September 6, 2023) and a research seminar after the project closing (National Emerging Infectious Diseases Laboratories (NEIDL), Boston University, Microbial Pathogenesis and Immunology Seminar Series held by the Department of Virology, Immunology & Microbiology at Boston University, Boston, USA.)

Science communication and media highlights
The significance of the project results is reflected in the fact that one of the publications was reviewed by one of the leading scientific journals, Science (Research Highlights, Vol 376. No 6593. In Other Journals, Caroline Ash et al.), and the editors of Nature Communications chose it as one of the best 50 newly published publications Microbiology category. We can also record an international success in science communication, as I was invited to the world's best-known podcast on virology, as the first Hungarian guest (This Week In Virology - TWiV 891: LLOV in the time of Ebola). The work had an echo in the international and domestic media, among others it was reviewed by MedicalXpress, Today UK News, Outbreak News Today, Laboratory News, The Conversation, 24.hu, index.hu.

Obstacles during the project and differences to the original plans:

- Due to the restrictions imposed by the COVID-19 pandemic, which limited our sampling capabilities, we narrowed our research focus to a single species and reduced the number of caves during the period of uncertainty regarding the reverse zoonotic potential of the SARS-CoV-2 virus. For multiple seasons we had to limit, or we had to cancel field sampling activities, following international recommendations of bat conservation.

- We encountered technical challenges in implementing mosquito trapping in caves, which led to delays in the research. As a result, we shifted our focus to other invertebrate vectors that might play a role in bat-to-bat transmission, such as bat flies and ticks, as previously mentioned. Additionally, we adopted a reverse strategy, attempting to detect the presence of viruses with potential insect vector origins in bat blood samples.

- Due to the substantial volume of data generated from next-generation sequencing (NGS) of bat blood – which is also an indicator for the successful experiment, as a vast amount of data were generated -, the bioinformatic analyses are still ongoing. The results from this research will be published after the conclusion of the grant period.

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2023.12.19.