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Národní plán obnovy

Příprava studijních materiálů studijního programu „Veterinární virologie“

Virové nákazy volně žijících zvířat ve vědě a výzkumu

Virové infekce související s netopýrytzv. „bat-borne“

Doktorský studijní program Veterinární virologie

Fakulta veterinárního lékařství

Veterinární univerzita Brno

Vytvoření doktorského studijního programu „Veterinární virologie“ na Veterinární univerzitě Brno
Specifický cíl B: Tvorba nových studijních programů v progresivních oborech

Projekt NPO registrační číslo NPO_VETUNI_MSMT-16594/2022

Výstup č. 2, vazba na cíl projektu č. 2, volitelný indikátor U3

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Virové nákazy volně žijících zvířat ve vědě a výzkumu

Virové infekce související s netopýry tzv. „bat-borne“

Jiří Pikula

Ústav ekologie a chorob zoozvířat, zvěře, ryb a včel
Veterinární univerzita Brno

Úvod: charakteristika netopýrů

Teplota těla létajícího netopýra - až 42 ° C

Tuto „**horečku**“ tolerují i viry cirkulující v populacích netopýrů

Zoonotické infekce pocházející od netopýrů probíhají u lidí se značnou **virulencí a patogenitou**

Vysoká horečka jako nespecifický obranný mechanismus je škodlivější pro nového hostitele (člověka) než pro virus

Miliony let koevoluce vztahu hostitel-patogen = **receptory** potřebné pro vstup netopýřích virů do buňky jsou přítomné i u ostatních skupin savců

Netopýři jsou rezervoárem infekcí, ale **klinicky manifestní onemocnění u nich pozorujeme spíše sporadicky**

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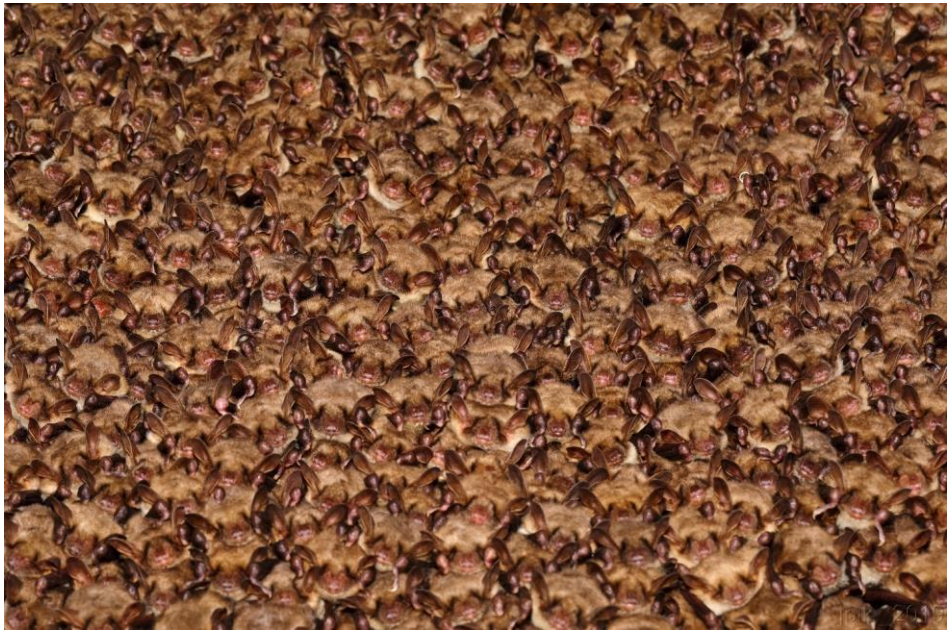
Faktory podporující cirkulaci patogenů v populacích netopýrů

- **Agregace** stovek až milionů jedinců
  - šíření patogenů **přímým/nepřímým kontaktem**



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Faktory podporující cirkulaci patogenů v populacích netopýrů

- Perzistence virových infekcí
- Přímý mezidruhový kontakt na koloniích

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Faktory podporující cirkulaci patogenů v populacích netopýrů

- Schopnost migračních přesunů
- Dlouhověkost (netopýr Brandtův 41 let)
- Hibernace a tzv. schopnost patogenů „přezimovat“ v hostiteli

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Faktory podporující cirkulaci patogenů v populacích netopýrů

- Netopýři mají nastaveny vysoké hladiny interferonu
  - Viry cirkulující u netopýrů na to musí být adaptovány

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Netopýři = **pokladnice patogenů**

Hrozba pro

Biodiverzitu netopýrů – *P. destructans*

Veřejné zdraví – zoonotická agens

Aktivní nebo pasivní surveillance

Synantropní druhy

- Znamená to značně rozdílné riziko expozice pro širokou veřejnost ve srovnání s chiropterology, výzkumníky a pracovníky s netopýry v záchranných stanicích

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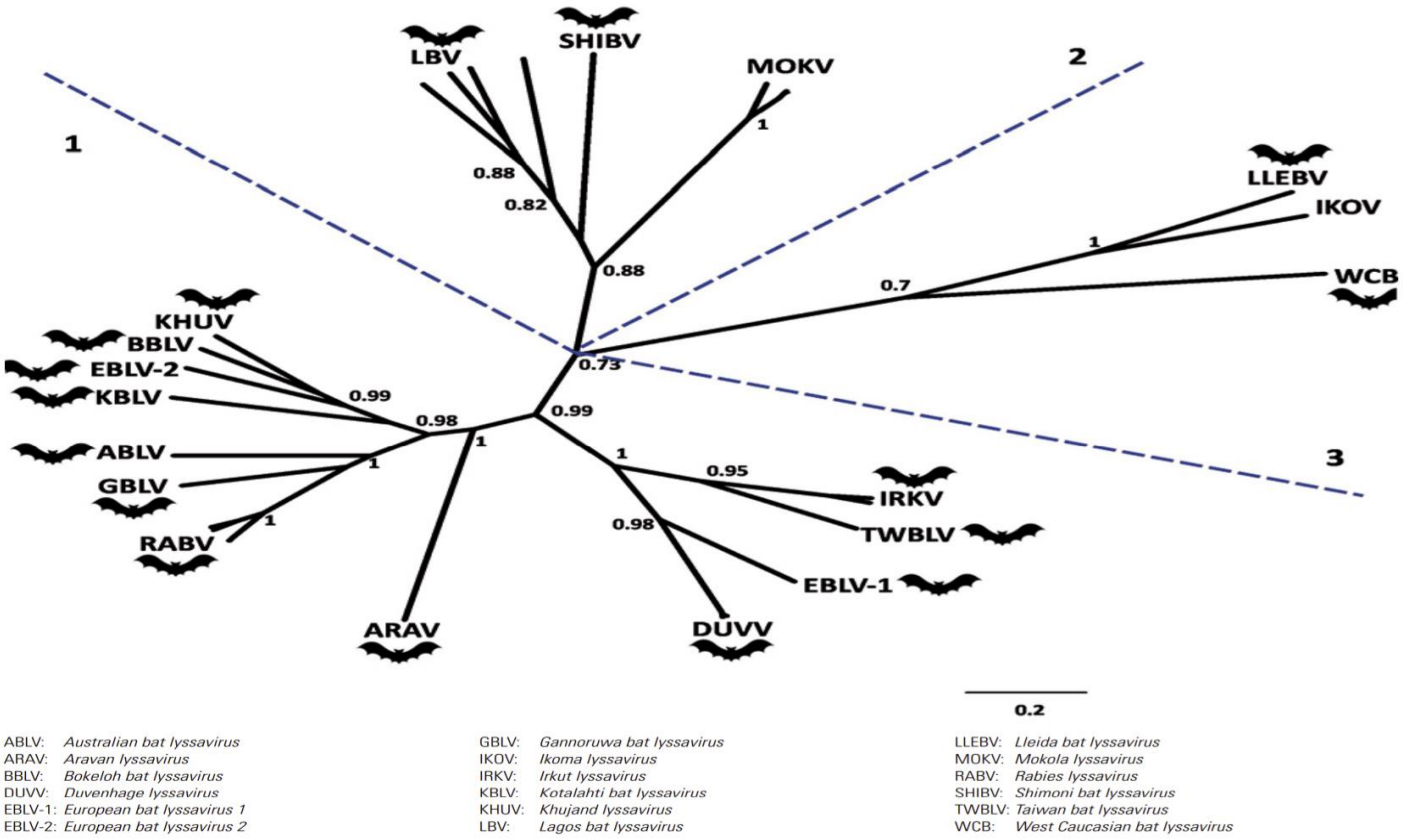
Lyssavirové infekce netopýrů

Markotter W, Coertse J (2018): Bat lyssaviruses. Revue scientifique et technique (International Office of Epizootics) 37(2): 385-400.



ABLV: *Australian bat lyssavirus*
ARAV: *Aravan lyssavirus*
BBLV: *Bokeloh bat lyssavirus*
DUVV: *Duvenhage lyssavirus*
EBLV-1: *European bat lyssavirus 1*
EBLV-2: *European bat lyssavirus 2*
GBLV: *Gannoruwa bat lyssavirus*
IKOV: *Ikoma lyssavirus*
IRKV: *Irkut lyssavirus*
KBLV: *Kotalahti bat lyssavirus*
KHUV: *Khujand lyssavirus*
LBV: *Lagos bat lyssavirus*
LLEBV: *Lleida bat lyssavirus*
MOKV: *Mokola lyssavirus*
RABV: *Rabies lyssavirus*
SHIBV: *Shimoni bat lyssavirus*
TWBLV: *Taiwan bat lyssavirus*
WCB: *West Caucasian bat lyssavirus*

Fig. 1
Phylogenetic reconstruction by Bayesian inference of all lyssaviruses, based on the first 480 nucleotides of the nucleoprotein gene
Node numbers indicate posterior probabilities. Dotted lines and numbers 1–3 represent phylogroups

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Lyssavirové infekce netopýrů

- Netopýři hlavním reservoárem většiny známých lyssavirů
- Značná seroprevalence antirabických protilátek u netopýrů
  - Zdá se, že netopýři jsou schopni přežít přirozenou infekci lyssaviry
    - Pravděpodobně díky „**abortivní periferní infekci**“
  - Spekuluje se také o virusonosičství s dlouhodobým vylučováním viru do prostředí bez projevu nervových příznaků
  - Možností je i přenos infekce jinak než pokousáním

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Lyssavirové infekce netopýrů

První pozitivní netopýr rozpoznán v Evropě 1954

Více než 400 pozitivních netopýrů detekováno v Evropě

2005, Praha – pozitivní *E. serotinus*

První případ vztekliny člověka po kontaktu s pozitivním netopýrem – 1977, Luhansk, Ukrajina

1985, Helsinki, Finsko – zemřel na vzteklinu švýcarský chiropterolog

2002, Skotsko – zemřel na vzteklinu po pokousání netopýry pracovník záchranné stanice

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PLOS ONE

Establishment of *Myotis myotis* Cell Lines - Model for Investigation of Host-Pathogen Interaction in a Natural Host for Emerging Viruses

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Abstract

Bats are found to be the natural reservoirs for many emerging viruses. In most cases, severe clinical signs caused by such virus infections are normally not seen in bats. This indicates differences in the virus-host interactions and underlines the necessity to develop natural host related models to study these phenomena. Due to the strict protection of European bat species, immortalized cell lines are the only alternative to investigate the innate anti-virus immune mechanisms. Here, we report about the establishment and functional characterization of *Myotis myotis* derived cell lines from different tissues: brain (*Mm*Br), tonsil (*Mm*To), peritoneal cavity (*Mm*Pca), nasal epithelium (*Mm*Nep) and nervus olfactorius (*Mm*Nol) after immortalization by SV 40 large T antigen. The usefulness of these cell lines to study antiviral responses has been confirmed by analysis of their susceptibility to lyssavirus infection and the mRNA patterns of immune-relevant genes after poly I:C stimulation. Performed experiments indicated varying susceptibility to lyssavirus infection with *Mm*Br being considerably less susceptible than the other cell lines. Further investigation demonstrated a strong activation of interferon mediated antiviral response in *Mm*Br contributing to its resistance. The pattern recognition receptors: RIG-I and MDA5 were highly up-regulated during rabies virus infection in *Mm*Br, suggesting their involvement in promotion of antiviral responses. The presence of CD14 and CD68 in *Mm*Br suggested *Mm*Br cells are microglia-like cells which play a key role in host defense against infections in the central nervous system (CNS). Thus the expression pattern of *Mm*Br combined with the observed limitation of lyssavirus replication underpin a protective mechanism of the CNS controlling the lyssavirus infection. Overall, the established cell lines are important tools to analyze antiviral innate immunity in *M. myotis* against neurotropic virus infections and present a valuable tool for a broad spectrum of future investigations in cellular biology of *M. myotis*.

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Národní plán obnovy

pathogens

MDPI

Review

Phylogeographic Aspects of Bat Lyssaviruses in Europe: A Review

Heliana Dundarova [1,*], Nadya Ivanova-Aleksandrova [2], Sarka Bednarikova [3], Irina Georgieva [2], Krasimir Kirov [4], Kalina Miteva [1], Boyko Neov [1], Peter Ostoich [1], Jiri Pikula [3], Jan Zukal [5] and Peter Hristov [1]

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[3] Department of Ecology and Diseases of Zoo Animals, Game, Fish and Bees, University of Veterinary Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic
[4] Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tzar Assen Str., 4000 Plovdiv, Bulgaria
[5] Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic
* Correspondence: heliana.dundarova@iber.bas.bg

Abstract: During the last few decades, bat lyssaviruses have become the topic of intensive molecular and epidemiological investigations. Since ancient times, rhabdoviruses have caused fatal encephalitis in humans which has led to research into effective strategies for their eradication. Modelling of potential future cross-species virus transmissions forms a substantial component of the recent infection biology of rabies. In this article, we summarise the available data on the phylogeography of both bats and lyssaviruses in Europe and the adjacent reg ions, especially in the contact zone between the Palearctic and Ethiopian realms. Within these zones, three bat families are present with high potential for cross-species transmission and the spread of lyssaviruses in Phylogroup II to Europe (part of the western Palearctic). The lack of effective therapies for rabies viruses in Phylogroup II and the most divergent lyssaviruses generates impetus for additional phylogenetic and virological research within this geographical region.

Harazim et al. BMC Immunology (2023) 24:7
https://doi.org/10.1186/s12865-023-00542-7

BMC Immunology

RESEARCH ARTICLE Open Access

Transcriptomic responses of bat cells to European bat lyssavirus 1 infection under conditions simulating euthermia and hibernation

Markéta Harazim[1,2*], Juliette Perrot[3], Hugo Varet[4], Hervé Bourhy[3], Julien Lannoy[3], Jiri Pikula[5], Veronika Seidlová[5], Laurent Dacheux[3†] and Natália Martínková[1,6†]

Abstract

Background Coevolution between pathogens and their hosts decreases host morbidity and mortality. Bats host and can tolerate viruses which can be lethal to other vertebrate orders, including humans. Bat adaptations to infection include localized immune response, early pathogen sensing, high interferon expression without pathogen stimulation, and regulated inflammatory response. The immune reaction is costly, and bats suppress high-cost metabolism during torpor. In the temperate zone, bats hibernate in winter, utilizing a specific behavioural adaptation to survive detrimental environmental conditions and lack of energy resources. Hibernation torpor involves major physiological changes that pose an additional challenge to bat-pathogen coexistence. Here, we compared bat cellular reaction to viral challenge under conditions simulating hibernation, evaluating the changes between torpor and euthermia.

Results We infected the olfactory nerve-derived cell culture of *Myotis myotis* with an endemic bat pathogen, European bat lyssavirus 1 (EBLV-1). After infection, the bat cells were cultivated at two different temperatures, 37 °C and 5 °C, to examine the cell response during conditions simulating euthermia and torpor, respectively. The mRNA isolated from the cells was sequenced and analysed for differential gene expression attributable to the temperature and/or infection treatment. In conditions simulating euthermia, infected bat cells produce an excess signalling by multitude of pathways involved in apoptosis and immune regulation influencing proliferation of regulatory cell types which can, in synergy with other produced cytokines, contribute to viral tolerance. We found no up- or down-regulated genes expressed in infected cells cultivated at conditions simulating torpor compared to non-infected cells cultivated under the same conditions. When studying the reaction of uninfected cells to the temperature treatment, bat cells show an increased production of heat shock proteins (HSPs) with chaperone activity, improving the bat's ability to repair molecular structures damaged due to the stress related to the temperature change.

Conclusions The lack of bat cell reaction to infection in conditions simulating hibernation may contribute to the virus tolerance or persistence in bats. Together with the cell damage repair mechanisms induced in response to hibernation, the immune regulation may promote bats' ability to act as reservoirs of zoonotic viruses such as lyssaviruses.

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Seidlova et al. BMC Veterinary Research (2020) 16:482
https://doi.org/10.1186/s12917-020-02702-y

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Active surveillance for antibodies confirms circulation of lyssaviruses in Palearctic bats

Veronika Seidlova[1*], Jan Zukal[2,3], Jiri Brichta[1], Nikolay Anisimov[4], Grzegorz Apoznański[5], Hana Bandouchova[1], Tomáš Bartonička[3], Hana Berková[2], Alexander D. Botvinkin[6], Tomas Heger[1], Heliana Dundarova[7], Tomasz Kokurewicz[5], Petr Linhart[1], Oleg L. Orlov[4,8], Vladimir Piacek[1], Primož Presetnik[9], Alexandra P. Shumkina[10], Mikhail P. Tiunov[11], Frantisek Treml[12] and Jiri Pikula[1,13]

Abstract

Background: Palearctic bats host a diversity of lyssaviruses, though not the classical rabies virus (RABV). As surveillance for bat rabies over the Palearctic area covering Central and Eastern Europe and Siberian regions of Russia has been irregular, we lack data on geographic and seasonal patterns of the infection.

Results: To address this, we undertook serological testing, using non-lethally sampled blood, on 1027 bats of 25 species in Bulgaria, the Czech Republic, Poland, Russia and Slovenia between 2014 and 2018. The indirect enzyme-linked immunosorbent assay (ELISA) detected rabies virus anti-glycoprotein antibodies in 33 bats, giving an overall seroprevalence of 3.2%. Bat species exceeding the seroconversion threshold included *Myotis blythii, Myotis gracilis, Myotis petax, Myotis myotis, Murina hilgendorfi, Rhinolophus ferrumequinum* and *Vespertilio murinus*. While *Myotis* species (84.8%) and adult females (48.5%) dominated in seropositive bats, juveniles of both sexes showed no difference in seroprevalence. Higher numbers tested positive when sampled during the active season (10.5%), as compared with the hibernation period (0.9%). Bat rabies seroprevalence was significantly higher in natural habitats (4.0%) compared with synanthropic roosts (1.2%). Importantly, in 2018, we recorded 73.1% seroprevalence in a cave containing a *M. blythii* maternity colony in the Altai Krai of Russia.

Conclusions: Identification of such "hotspots" of non-RABV lyssavirus circulation not only provides important information for public health protection, it can also guide research activities aimed at more in-depth bat rabies studies.

Keywords: Chiroptera, rabies, blood samples, seroprevalence, Europe, Siberia

Materiál a metodika

- Neletální odběr vzorků krve v Bulharsku, ČR, Polsku, Rusku a Slovinsku v letech 2014 až 2018

- 1027 netopýrů 25 různých druhů

- Serologické testy (ELISA validovaná FAVN)
  - rabies virus anti-glycoprotein antibodies
  - fluorescent antibody virus neutralization

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Výsledky

- Celková seroprevalence vztekliny netopýrů **3,2 %**

- **Pozitivní druhy**
  - *Myotis blythii*, *Myotis gracilis*, *Myotis petax*, *Myotis myotis*, *Murina hilgendorfi*, *Rhinolophus ferrumequinum*, *Vespertilio murinus*

- „hotspot“ **73,1 %** seroprevalence v jeskyni s mateřskou kolonií *M. blythii*

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Hendra

Paramyxoviridae, rod Henipavirus

- Fatální respirační a nervová infekce koní a lidí
- 1994, Austrálie
- Koně se nakazí virem v moči infikovaných kaloňů (*Pteropus*)
- Člověk se nakazí kontaktem s pozitivním koněm
- Nedochází k šíření z člověka na člověka

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Nipah

- Nákaza prasat v Malajsii a Singapuru (poprvé 1999)
    - dále Bangladéš a Indie
- Šíří se kontaktem s močí a slinami
- Rezervoárem jsou kaloni rodu *Pteropus*

- Po průniku do populace lidí se dále může šířit z člověka na člověka
- Vykazuje mortalitu 40-70 % v důsledku encefalitidy

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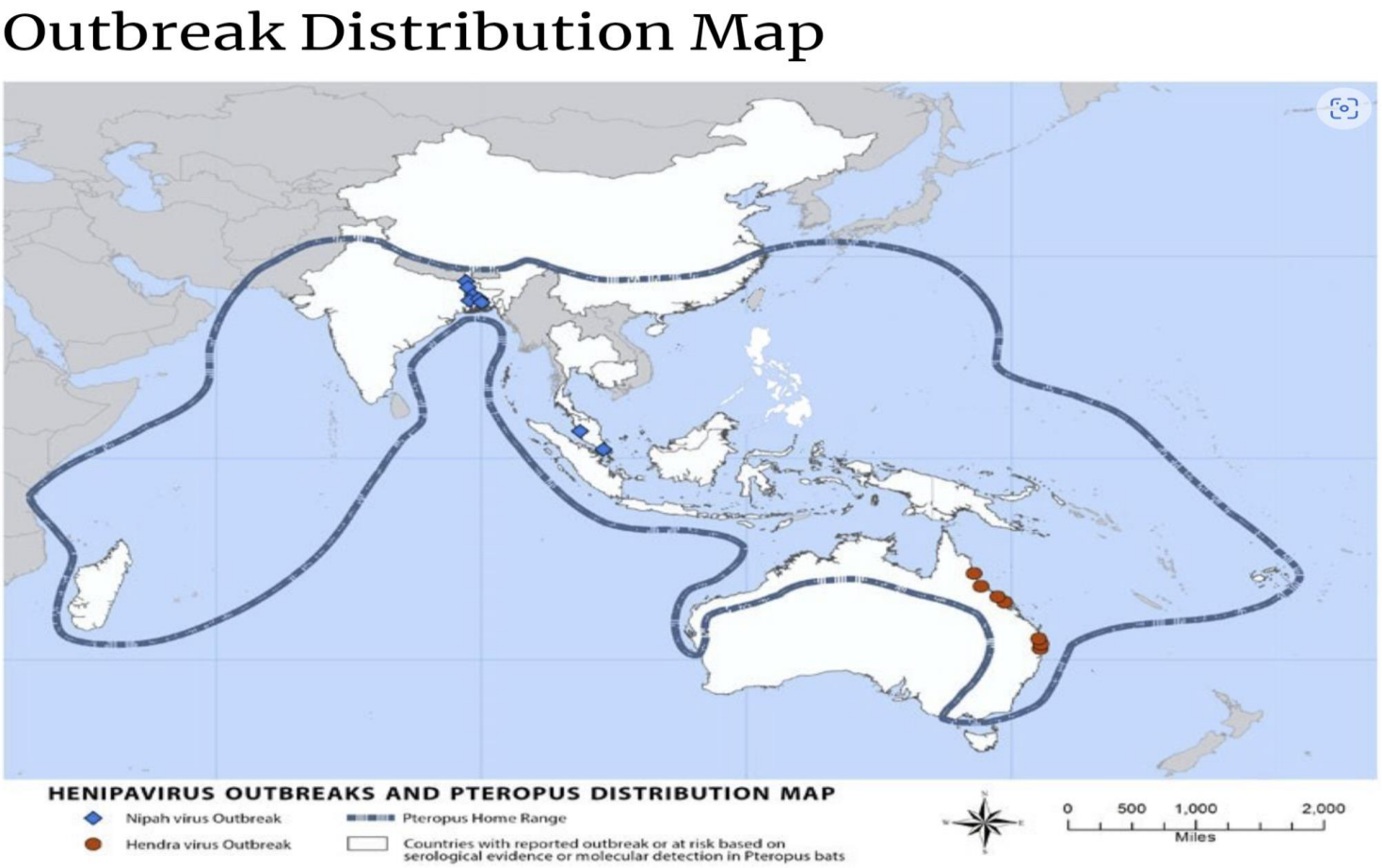
Hendra a Nipah



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Filoviry

Ebola a Marburg

- přirozené infekce/endemický výskyt v sub-Saharské Africe
- bez klinicky manifestního onemocnění u netopýrů

Negredo A et al. (2011): Discovery of an Ebolavirus-Like Filovirus in Europe. PLOS Pathogens 7(10): e1002304.

- Hromadné úhyny létavce stěhovavého (*Miniopterus schreibersii*) v roce 2002 ve Francii, Španělsku a Portugalsku
- Virová pneumonie

2013, úhyn 500 zvířat v Maďarsku

Kemenesi G et al. (2018): Re-emence of Lloviu virus in *Miniopterus schreibersii* bats, Hungary, 2016. Emerging Microbes & Infections 7(1): 1-4.

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Koronaviry (SARS-CoV)

Severe Acute Respiratory Syndrome

- 2002, netopýři a kaloni
- čínská provincie Kuang-tung
- Respirační infekce + cytokinová bouře
- Smrtnost 9,6 %

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Koronaviry (MERS-CoV)

Middle East Respiratory Syndrome

- 2012, netopýři a velbloudi
- Respirační infekce + selhání ledvin u lidí
- Smrtnost 30 %

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Koronaviry (SARS-CoV-2)

Netopýři jsou rezervoárem i pravděpodobným zdrojem SARS-CoV-2

Ochrana netopýrů – zákaz terénní práce, aby se infekce nepřenesla z lidí na netopýry

Chybí poznatky o interakci mezi koronaviry a netopýří buňkou

Zkoumali jsme schopnost viru SARS-CoV-2 replikovat se v primárních a imortalizovaných buněčných kulturách derivovaných z netopýrů *Rhinolophus ferrumequinum*, *Myotis myotis*, *Eptesicus serotinus*, *Tadarida brasiliensis* a *Nyctalus noctula*

Permisivita netopýřích buněk pro koronavirovou infekci je minimální, dokonce i u buněk, které exprimují detekovatelné množství virového receptoru ACE2 (angiotenzin-konvertující enzym 2)

Rezistenci k infekci lze překonat v buněčných kulturách vložením a exprimací lidského ACE2 (hACE2), což svědčí o tom, že restrikce replikace je dána nízkou exprimací netopýří ACE2 nebo absencí vazebného místa na těchto buňkách

Schopnost netopýřích buněk účinně regulovat virovou replikaci je dána **silnou produkcí interferonu**

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Koronaviry (SARS-CoV-2)

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VIRUS-CELL INTERACTIONS

Species-Specific Molecular Barriers to SARS-CoV-2 Replication in Bat Cells

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ABSTRACT Bats are natural reservoirs of numerous coronaviruses, including the potential ancestor of SARS-CoV-2. Knowledge concerning the interaction between coronaviruses and bat cells is sparse. We investigated the ability of primary cells from *Rhinolophus* and *Myotis* species, as well as of established and novel cell lines from *Myotis myotis*, *Eptesicus serotinus*, *Tadarida brasiliensis*, and *Nyctalus noctula*, to support SARS-CoV-2 replication. None of these cells were permissive to infection, not even the ones expressing detectable levels of angiotensin-converting enzyme 2 (ACE2), which serves as the viral receptor in many mammalian species. The resistance to infection was overcome by expression of human ACE2 (hACE2) in three cell lines, suggesting that the restriction to viral replication was due to a low expression of bat ACE2 (bACE2) or the absence of bACE2 binding in these cells. Infectious virions were produced but not released from hACE2-transduced *M. myotis* brain cells. *E. serotinus* brain cells and *M. myotis* nasal epithelial cells expressing hACE2 efficiently controlled viral replication, which correlated with a potent interferon response. Our data highlight the existence of species-specific and cell-specific molecular barriers to viral replication in bat cells. These novel chiropteran cellular models are valuable tools to investigate the evolutionary relationships between bats and coronaviruses.

IMPORTANCE Bats are host ancestors of several viruses that cause serious disease in humans, as illustrated by the ongoing SARS-CoV-2 pandemic. Progress in investigating bat-virus interactions has been hampered by a limited number of available bat cellular models. We have generated primary cells and cell lines from several bat species that are relevant for coronavirus research. The various permissivities of the cells to SARS-CoV-2 infection offered the opportunity to uncover some species-specific molecular restrictions to viral replication. All bat cells exhibited a potent entry-dependent restriction. Once this block was overcome by overexpression of human ACE2, which serves at the viral receptor, two bat cell lines controlled well viral replication, which correlated with the inability of the virus to

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Bats as another potential source of murine gammaherpesvirus 68 (MHV-68) in nature

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Keywords: MHV-68; antibody; bats; virus ecology; herpesvirus

Murine gammaherpesvirus 68 (MHV-68) is a natural pathogen of free-living murid rodents. In 1976, MHV-68 was isolated from a bank vole *Clethrionomys glareolus / Myodes glareolus* captured near Bratislava, Slovakia (*1*). Based on molecular analysis, the virus was classified to the species *Murid herpesvirus 4* (MuHV-4), the genus *Rhadinovirus*, the subfamily *Gammaherpesvirinae*, the family *Herpesviridae* and the order *Herpesvirales* (*2*).

Genome of this virus was sequenced by Virgin in 1997 (*3*). MHV-68 is an accepted animal model for the investigation of pathogenesis, oncogenesis and immunology of human oncogenic gammaherpesviruses (*4, 5*). Based on the recent ecological studies it is known that this virus may spread from its reservoir wild animals to other animal species in the same biotope as well as to livestock and household animals (*6*). The role of individual animal species in MHV-68 infection is not known. It is widely known that host-switching of a virus can have fatal consequences for the new host. Presence of serum antibodies to MHV-68 was detected in various hosts from wild reservoir (wood mouse, bank vole, field vole, yellow-necked mouse, wild mouse) or non-reservoir animals (wild boar, red fox, fallow deer, red deer, European roe deer, hare), to farm, domestic and household animals (goat, horse, cattle, dog, cat, wild house mouse), to humans (laboratory personnel working with the virus, hunters, people coming into contact with forest animals) and vectors (ticks) (*6*). Antibodies were assayed by virus neutralization assay, complement fixation test or ELISA. The presence of viral DNA in some of the samples was also confirmed by PCR.

Bats are intensively studied animals since they have been confirmed as a reservoir for many viruses, such as rabies virus or tick-borne encephalitis virus. These viruses are potentially dangerous to humans. Viruses detected in bats can also cause severe viral infection in the human population, often with fatal consequences, e.g. SARS coronavirus, MERS coronavirus, Ebola virus, Marburg virus, Hendra virus, Nipah virus. Currenty, there is only limited information on herpesviruses and bats, and while all three subfamilies of herpesviruses have been detected in bats around the world, their biology is not well studied (*7, 8, 9*).

The aim of presented study was to look for the presence of MHV-68 in blood samples from bats using serological and direct detection methods.

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SHORT COMMUNICATION

No Virological Evidence for an Influenza A - like Virus in European Bats

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Impacts

- Bats have been identified a 'treasure trove' of new mammalian virus species.
- New influenza A virus subtypes have been detected in bats from South America.
- No evidence, by molecular investigations, for the occurrence of such influenza viruses was found in bats from Central Europe.

Keywords:
Influenza; infectious disease; surveillance; bats

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Samples collection:
Friedrich-Loeffler-Institute, Riems, Germany, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, Romanian Bat Protection Association, Satu Mare, Romania

Summary

New members of the influenza A virus genus have been detected recently in bats from South America. By molecular investigations, using a generic real-time RT-PCR (RT-qPCR) that detects all previously known influenza A virus subtypes (H1-H16) and a newly developed RT-qPCR specific for the South American bat influenza-like virus of subtype H17, a total of 1571 samples obtained from 1369 individual bats of 26 species from Central Europe were examined. No evidence for the occurrence of such influenza viruses was found. Further attempts towards a more comprehensive evaluation of the role of bats in the ecology and epidemiology of influenza viruses should be based on more intense monitoring efforts. However, given the protected status of bats, not only in Europe, such activities need to be embedded into existing pathogen-monitoring programs.

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OPEN

A common partitivirus infection in United States and Czech Republic isolates of bat white-nose syndrome fungal pathogen *Pseudogymnoascus destructans*

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The psychrophilic (cold-loving) fungus *Pseudogymnoascus destructans* was discovered more than a decade ago to be the pathogen responsible for white-nose syndrome, an emerging disease of North American bats causing unprecedented population declines. The same species of fungus is found in Europe but without associated mortality in bats. We found *P. destructans* was infected with a mycovirus [named Pseudogymnoascus destructans partitivirus 1 (PdPV-1)]. The virus is bipartite, containing two double-stranded RNA (dsRNA) segments designated as dsRNA1 and dsRNA2. The cDNA sequences revealed that dsRNA1 dsRNA is 1,683 bp in length with an open reading frame (ORF) that encodes 539 amino acids (molecular mass of 62.7 kDa); dsRNA2 dsRNA is 1,524 bp in length with an ORF that encodes 434 amino acids (molecular mass of 46.9 kDa). The dsRNA1 ORF contains motifs representative of RNA-dependent RNA polymerase (RdRp), whereas the dsRNA2 ORF sequence showed homology with the putative capsid proteins (CPs) of mycoviruses. Phylogenetic analyses with PdPV-1 RdRp and CP sequences indicated that both segments constitute the genome of a novel virus in the family *Partitiviridae*. The purified virions were isometric with an estimated diameter of 33 nm. Reverse transcription PCR (RT-PCR) and sequencing revealed that all US isolates and a subset of Czech Republic isolates of *P. destructans* were infected with PdPV-1. However, PdPV-1 appears to be not widely dispersed in the fungal genus *Pseudogymnoascus*, as non-pathogenic fungi *P. appendiculatus* (1 isolate) and *P. roseus* (6 isolates) tested negative. *P. destructans* PdPV-1 could be a valuable tool to investigate fungal biogeography and the host–pathogen interactions in bat WNS.

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