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Caves as a reservoir for novel and reoccurring zoonoses - ecological monitoring and metagenomic analysis in real time

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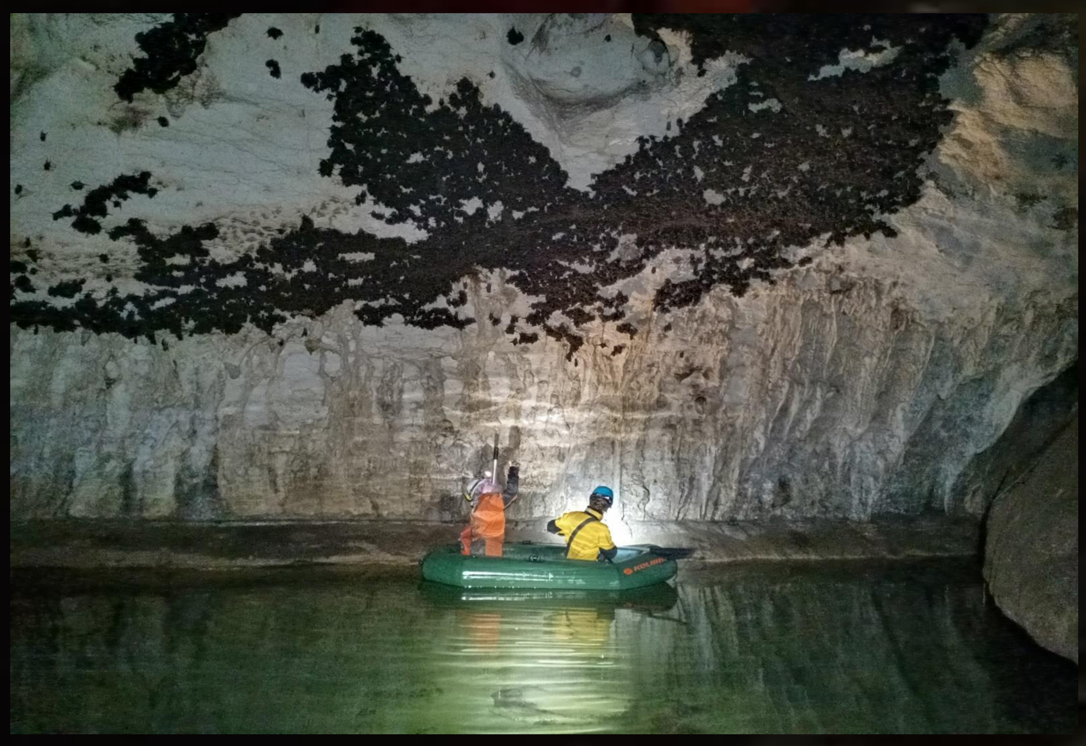
THE PROBLEMS

Zoonotic infectious diseases, or zoonoses, are bacterial, viral, or fungal diseases that can be transmitted between species, such as between humans and animals. More than half of all known infectious diseases are zoonotic, including the bubonic plague, the Spanish flu, HIV/AIDS, and, more recently, COVID-19

Over 30% of the deaths worldwide are due to infectious diseases and 75% of the emerging infectious diseases are zoonoses. We are contemporaries of an unprecedented "boom" in the discovery of new infectious diseases. Observations from recent years show that an average of one new agent appears each year and its origin is from animals. Emerging risks increase when, as a result of anthropogenic activity, abrupt changes in ecosystems occur intentionally or accidentally, leading to a disturbance of the balance between the pathogen and the usual host species.

Bats are the second largest order of mammals and a significant reservoir of viral, bacterial and fungal pathogens, some of which are particularly dangerous. Bats are known to carry more pathogens than any other mammal. The specific bat physiology and their environmental preferences modulate a microbial flora with unpredictable potential.



Caves are ecological niches with extreme conditions, where bats form colonies. Up to date there are no detailed studies which characterized the metagenomic diversity of cave dwellings (as bats and blood-sucking insects) and their environment (water and soil). Caves are extreme ecological niches, where various stress factors, such as high humidity, constant low temperature, darkness can shape the emergence of new pathogens.

PROJECT OBJECTIVES AND HYPOTHESES

The dynamically changing environment and climatic conditions will increasingly play a significant role in the spread of new and emerging infections in new geographical areas and ecological niches.
The project aims to develop a research model for the monitoring of viral, bacterial, fungal and protozoan new and emerging pathogens transmitted by insects and animal species inhabiting caves and actively in contact with the external environment. We aim to identify places of origin and spread of known and new pathogens for humans and animals. A model of research will be caves, which are specific ecological niches with extreme living conditions. Caves in which increased anthropogenic impact with close contacts between microbial species and their permanent or intermediate hosts (arthropods and bats) appearing reservoirs and propagation vectors will be studied. The unusual interaction of man with the cave dwellers, e.g. large bat colonies can lead to the formation of a chain with epidemic risk.
Our goal is to develop and test an approach for monitoring and predicting epidemic outbreaks of new and emerging pathogens in the environment in real time by applying complete genomic sequencing and bioinformatics analysis.
Several tasks will be performed to achieve the set goal:
1. Sampling in caves, examining blood and carcass material from bats, mosquitoes, water and soil.
2. Isolation of DNA from the samples and performing random (shotgun) complete genomic sequencing, aiming to identify all genomes present in the samples. Sequencing of 100-140 million nucleotides per sample, which is> 40 Gb of information.
3. Development of bioinformatics approaches for analysis, identification of biodiversity (anatomy of genomes) of viruses, bacteria, fungi and protozoa, interpretation and visualization of results.
4. Development of a model for predicting the occurrence of new and recurrent infections by assessing the pathogenic potential of the identified species in the samples in relation to the known information in the literature or databases for viral, microbial and protozoan pathogens in animals and humans.
The aim of the project is based on the hypotheses that: (*i.*) The microbiome of bats, bedbug mosquitoes and subterranean insect fauna can be a reliable indicator of the emergence and spread of new and emerging pathogens in real time.
The results we will obtain will allow for a more detailed description of the biodiversity in the caves and to assess the potential of this biodiversity for the formation and emergence of future epidemics.
We accept (ii) the hypothesis that complete genomic (shotgun) sequencing for analysis of animal samples and from the environment is a more informative and reliable approach to research than the now widely used target sequencing to identify microbial biodiversity by 16S rDNA, ITS and 18S rDNA marker genes.

THE TEAM

The project is an example of close cooperation between clinical microbiologists, molecular biologists, zoologists, speleologist and bioinformatics specialists in the name of a goal that each team would not decide individually. The combination of knowledge of speleology, medical entomology, microbiology, molecular biology, histology and bioinformatics makes the research of interdisciplinary significance. Along with complex interdisciplinarity, the research approach aims at a reasonable realism of the research.

The National Museum of Natural History - Bulgarian Academy of Sciences, Sofia, is the place where biospeleological research is born and where concentrations are currently concentrated in Bulgaria. The existing Center for Biospeleology stores a huge number of materials and data from the research of the caves in our country, which lasted for over 90 years. All museum experts involved in the project have vast experience in researching underground habitats and speciesThe team members are qualified cavers, and one third of the team is qualified cave rescuers, part of Cave Rescue - Bulgaria, which is a huge advantage in light of the specific requirements for biospeleological research.

The National Center of Infectious and Parasitic Diseases (NCIPD) team is showing a lasting interest in studying the human microbiome and suspected latent infectious agents. In the last few years, a successful strategy for the analysis of the blood microbiome has been developed - the so-called complete microbial resuscitation / recovery by blood culture, microscopy and next generation DNA sequencing. Among cultured and uncultivated blood samples, the team identified a large biodiversity of OTU (Operational Taxonomic Unit) belonging to 6 types (phylum) and 15 bacterial orders (ordo), with Proteobacteria of the genus predominating. Types (phylum) Ascomycota and Basidiomycota predominate among the proven fungi.

ANY FUTURE COLLABORATION IS WELCOME!

OUR FIRST RESULTS:

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Toshkova N, Zlatkov B, Fakirova A, Zhelyazkova V, Simov N (2022) First record of *Psorergatoides* Fain, 1959 (Acari, Cheyletoidea, Psorergatidae) for the Balkan Peninsula with description of the cutaneous lesions on the wing membrane of its hosts *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii* (Tomes, 1857) (Chiroptera, Vespertilionidae). Biodiversity Data Journal 10: e89514. https://doi.org/10.3897/BDJ.10.e89514

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