

INTO THE MICROBIAL LIFE OF THE CAVES; PATHOGENS AWAITING IN THE UNDERGROUND

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Abstract

Caves frequently visited by animals or humans present reservoirs of pathogenic or conditioned pathogenic microorganisms. In recent years, numerous bacteria, fungi and viruses have been isolated and identified from caves as pathogens for humans and animals. Furthermore, through humans and/or animals, new types of germs can be carried into the normal microflora of caves, disturbing the microbial balance, making these locations sources of pathogenic germs with zoonotic potential. The most common diseases caused by microorganisms in caves are diseases located in the respiratory system. Visits to any underground environment should no longer be seen as a simple, risk-free tourist activity, but rather as one with potential risks to human and animal health. Numerous studies have been conducted worldwide on the diversity and abundance of pathogens in caves. In order to reduce the number of diseases associated with caves, it is necessary to raise awareness and educate about the possible dangers to people or animals that come in direct contact with such environments.

Key words: cave, microbiome, pathogens.

INTRODUCTION

Caves frequently visited by humans or populated by animals are reservoirs of pathogenic microorganisms. In recent years, many bacteria, fungi and viruses in caves have shown pathogenic potential for humans and animals, especially in the tropics. The zoonotic character of these pathogens is still debatable as variations of these viruses or bacteria have specific targets. For instance, historic pandemics such as MERS-CoV or SARS-CoV-2 have reached the assumption that coronaviruses from dromedary camels or bats have suffered mutations that allowed them to cause highly dangerous respiratory syndromes in humans. Although it is difficult to associate a specific infection with a single visit to an underground environment, several studies indicate that visits to caves and mining areas have resulted in fatal diseases.

Microbial life of the caves

The globe's microorganism population is extremely diverse and varied, with many specimens still an enigma to humans. These microorganisms occupy all stages of the

biosphere, including the subterranean areas. Underground habitats are mainly caves, with little or no light input, with few resources of organic nutrients, relatively constant temperature and areas extracted from mineral surfaces. Specialized studies on microbial communities in karst environments are limited, most being conducted in caves in Spain, Italy, Romania or the United States. However, these studies focus on specific issues, such as taxonomy or geomicrobiology (Hernandez-Marine and Canals, 1994).

More recent studies try to highlight the biodiversity and limited distribution of bacteria in the karst environment. *Proteobacteria* represent the majority of bacteria found in caves, followed by representatives of the genus *Actinobacteria* (Barton et al., 2007). Bacteria of the genus *Actinobacteria* have been attributed mainly to soils, which represent their natural habitat, but in the last decade they have been shown to be frequently associated with the karstic environment (Schabereiter-Gurtner et al., 2002). This points out that for this group of bacteria, the microclimate conditions and the nutrient sources of the caves represent a favorable habitat.

Microclimate conditions and high salinity create certain niches and stability in microbial communities, and this leads to increased interest in in-depth studies in order to understand the natural processes that make up these microbial communities. In addition, the interaction of the composition of the karst environment, both biological and mineral, determines the biodiversity of these ecosystems, still insufficiently known.

These previously mentioned groups are very widespread in caves, and the description of new species is in a continuous flow of development due to the new molecular methods that have emerged. These methods have the advantage of taxonomically classifying the subspecies of these microorganisms. For example, after the emergence of new research methods, the genus *Mycobacterium* includes over 130 species (Mignard and Flandrois, 2008), the genus *Nocardia*, about 70 species (Rodríguez-Nava et al., 2008), the genus *Gordonia* 21 species (Blanc et al., 2007), genus *Rhodococcus* 32 species, *Brevibacterium* with 19 species and *Micrococcus*, 9 species.

Pathogens awaiting in the underground

Certain bacteria of this genus are responsible for various lung, brain or skin infections in humans.

Species such as *G. bronchialis*, *G. otitidis*, *G. aichiensis*, and *G. terrae* of the genus *Gordonia* are described in the literature as opportunistic pathogens responsible for bacteremia and bronchopulmonary disease (Iida et al., 2005; Blanc et al., 2007). Such species have been isolated and identified from the Grotta dei Cervi cave, located in Italy, but the possible variety of this habitat is not yet fully established. The genus *Gordonia* is of great interest in the field of biotechnology due to its ability to degrade and bioremediate sulfur from fuels (Lee et al., 2005).

The main species of the genus *Rhodococcus* are represented by *R. equi* and *R. erythropolis* potentially pathogenic bacteria, isolated from caves located in northern Spain. (Vernazza et al., 1991) (Groth et al., 1999). Regarding the genera *Brevibacterium* and *Micrococcus*, they have been little studied, and their involvement in clinical cases is rarely reported in the literature. Species such as *B. casei* or *B.*

epidermis are normally part of the human skin flora, being responsible only for opportunistic infections (Reinert et al., 1995). *Micrococcus luteus* is also responsible for certain common diseases in people with immunodeficiency (Salar et al., 1997). Reverend to the *Streptomyces* family, the main pathological agent with potential pathogen is *Streptomyces somaliensis*, an accidental etiologic agent of actinomycetoma in countries such as India or Sudan (Nasher et al., 1989).

The growing interest in such ecosystems is not strictly due to the diversity and taxonomic uncertainty of these bacteria, but more importantly, the role that these microorganisms could play on human and animal health. The potential pathogenic or even zoonotic risk of these bacteria coming from natural ecological niches, in this case, the underground ones, can be a real problem in human and veterinary health.

Evidence of a considerable reservoir of pathogenic bacteria present in tourist karst environments, namely species belonging to the *Proteobacteria* cluster, has also been reported. Thus, a study conducted in caves in Spain revealed the presence of a species of *Alphaproteobacteria*, called *Inquilinus limosus*, which has been implicated in the occurrence of cystic fibrosis in humans (Schmoltdt et al., 2006). Studies in this direction have reported the presence of *Aurantimonas altamirensis*, a bacterium of the genus *Alphaproteobacteria*, in the Altamira Cave (Jurado et al., 2006). The bacterium was later isolated in a Canadian hospital from three patients diagnosed with cystic fibrosis, keratitis, and corneal ulcer (Luong et al., 2008).

Also, certain species of the genus *Afipia* have been associated with protozoa, and their role is suspected in the occurrence of nosocomial diseases (La Scola et al., 2002). With the help of molecular techniques, *Legionella* reservoirs have also been detected in caves in France (Bastian et al., 2009). In addition, bacteria such as *Staphylococcus aureus* have recently been isolated by researchers from karst environments of tourist value, highlighting the possible human impact. This type of bacteria is frequently associated with nosocomial infections in countries such as India or the USA (Mendes et al., 2009).

Spirochetes are also frequently associated with karst environments due to the favorable survival conditions that these habitats offer. Rodents and bats are reservoirs of leptospirosis in many tropical caves, which maximize the exposure of tourists to this disease. High heat and humidity can cause tourists to choose minimal clothing protection, and frequent contact with cave rocks can result in multiple skin microlesions. These wounds are often the main route of infection in leptospirosis (Mortimer, 2005).

Another condition commonly associated with caves in some countries is recurrent borreliosis fever. Ticks are the basic vectors of *Borrelia* spp. And they are widespread in India, Iran, Syria, Turkey, Cyprus, Egypt. In Israel, about 40-50% of caves have been found to be infested with *Ornithodoros tholozani*, the most important vector for *Borrelia persica*, the cause of recurrent tick-borne fever. *O. tholozani* parasitizes rodents and small mammals, which are the natural reservoir of the bacterium.

These bacteria, which are isolated from caves and have a significant pathogenic potential, pose a real danger to humans and animals, especially at-risk groups. However, the virulence, pathogenicity and spread of these species of microorganisms are not always constant in the strains. This suggests that the strains were isolated from niches with different characteristics, thus acquiring and developing different pathogenic factors. Also, the fact that there are few clinical observations on these microorganisms may give the false impression that only a few strains are pathogenic. This could be due to: a) relatively new detection methods, as it refers to a recently described species, b) low environmental incidence, c) the quality of an opportunistic pathogen that requires special conditions of the host's immune system. On the other hand, it would also be interesting to obtain information on the infectious capacity of strains isolated from caves through experimental research, studying their virulence capabilities with PCR methods, using virulence genes as markers and by interaction tests with other hosts (amoebae, fungi, insects, etc.).

There is a high risk, due to the presence of humans in caves, of a bacterial passage from humans to caves that can lead to the

development of new lines of bacteria with different levels of pathogenicity. This phenomenon can be assessed by measuring human indicator bacteria. On the other hand, adaptation factors after a strain exchange (from human to cave and vice versa) can be recognized by transcriptome and comparative genomic studies.

Microbiological studies performed from the air of the Magurici cave, showed that the total number of germs varied between 102 and 104 cfu/m³ of air. Gram-negative bacteria and staphylococci ranged from 0 to 102 cfu/m³, and streptococcal and fungal values ranged from 5 × 10 to 103 cfu/m³ air. The prevalence for these bacteria showed maximum values during the summer, close to the maternity period of the bats. The microbiological values observed during the time are comparable to those observed in the Central Park of Cluj-Napoca (NTG = 629-10479 cfu/m³; G- = 0-786 cfu/m³; SP = 0-1402 cfu/m³; ST = 314- 1572 cfu/m³; Fungi = 550-109970 cfu/m³). In spring, autumn and winter the values were similar to the microbiology of the air in the mountain resort Păltiniș (NTG = 366 cfu/m³; G - = 0 cfu/m³; SP = 0 cfu/m³; ST = 0 cfu/ m³; Fungi = 2907 ufc/m³) (Drăghici, 1982).

However, these values of microorganisms in the air can vary depending on the cave, the intensity of the search for food outside the habitat, the increase in maternity and most likely the number of dead youth. This bacterial contamination of the air can pose a risk to animals and humans (Hartung 1994). It is known that about 20% of infectious diseases have air as the main route of transmission, and areas with temperate climates, such as karst, are predisposing to such diseases (Manescu et al., 1993). Thermal circulation and ventilation thus play an important role in keeping the air clean, due to the fact that bats usually prefer well-ventilated areas in caves, for a cooler climate. Cave ventilation plays an important role in preventing air pollution and contributes to the health of bat populations. Bats being an inhabitant of caves but also one who often seeks nutrients in the outside world could easily carry and spread potential pathogenic agents (Borda et al., 2004).

CONCLUSIONS

Frequent visits to caves can not only lead to the emergence of disease outside of these environments but also disrupt the existing microbiome. Continuous changes in the prevalence of agents in caves may lead to the appearance of important zoonotic diseases, as proven by the 2019 coronavirus pandemic.

The bacterial microflora introduced in the underground environment can adapt to the new microclimate conditions and represent new sources of pathogenic germs or pathological conditions both for humans and for cave fauna (especially bats). Further research is needed to properly establish the possible dangers lurking in underground environments.

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