

HUMAN PATHOGENS AMONG BATS

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ABSTRACT

Bats are known to inhabit both caves and open space areas. Bulgaria is among the European countries with the highest number of bat species. The species found in the country are distributed over a wide area. They range from the Pyrenees and the British Isles to the Pacific region and the Far East. Many bat species are carriers of potential human pathogens. Bats play an important role in agriculture and act as a biological pest crop control agent. Bulgarian bat ecosystem comprises temperate climate and a wide range of abiotic factors, including humidity, darkness, sunlight, and temperature variations. The metabolism of bats and their body temperature vary significantly between the period of activity and the hibernation. Fluctuations in body temperature can potentially impact host microbiome biodiversity. Temperature variations may induce a high level of microbial mutagenesis. Additionally, the existence in large, mixed-species colonies, together with a relatively long individual lifespan (4 – 16 years) and extensive travel distances, enhances the likelihood of encountering multiple pathogens in a single host organism. This, in turn, facilitates genetic variations

and re-combinations among those microbial pathogens, thereby raising their potential to breach species barriers. In this review, we summarized and analyzed the available scientific information concerning the potential microbial human pathogens associated with bats, alongside with our preliminary data on the biodiversity of bats blood microbiome. Future research should focus on bats as both pathogens carriers and dynamic models for predicting emerging and re-emerging zoonotic diseases.

Key words: bats, virome, bacteriome, metagenomics, zoonoses, vector, ectoparasites

INTRODUCTION

Bats are the second biggest mammalian order after rodents comprising more than 1400 species or about 20% of the recent mammalian species. They are the only mammals capable of sustained flight. Taxonomically, bats are grouped in order Chiroptera. About 43 bat species are encountered in Bulgaria. Bulgaria stands out as one of the European countries with the highest diversity of bat species. The majority of European species are found in the country, with only a few exceptions including *Myotis escaleraei* Cabrera, 1904, *Myotis punicus* Felten, 1977, *Eptesicus isabellinus* Temminck, 1840 and *Rousettus aegyptiacus* (Geoffroy, 1840). In Bulgaria, three bat families are present: Rhinolophidae, Vespertilionidae, and Molossidae. (1).

Bats, known for their social and communicative nature, often live in large colonies (2). This social structure, along with their ability to fly over large distances potentiates a quicker spread of potential infectious diseases compared to many other mammal groups. Typically, bat colonies consist of various species, further facilitating the development of mechanisms by which microbes can overcome species barriers.

The body temperature of bats at rest is 38 °C, while in active flight it reaches 41 °C which resembles a state of infectious process in man. On the opposite, during the hibernation period, the body temperature of the animal drops to match that of the environment, typically around 6-12 °C (3). The specific bat physiology and their environmental preferences modulate a microbial flora with unpredictable potential.

In Bulgaria, as in most European countries bats are

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predominantly insectivorous. This diet exposes them to pathogens carried by insects, which can be potentially transmitted further up the food chain. Insect vectors can transmit infectious pathogens between humans and animals, or vice versa. Additionally, many biting and blood-sucking ectoparasites are spread by bats, such as ticks, lice, fleas, and flies. These parasites have the potential to act as reservoirs or secondary vectors for various pathogens.

Bats have a relatively long lifespan for their size, 4 years on the average, with exceptions up to 16 years (4,5), which provides a long timeframe for carrying and potentially spreading pathogens. This extended lifespan increases the period during which they can act as reservoirs and vectors of diseases and a site of potential genetic recombination (horizontal genetic transfer) between transmitted pathogens (6).

Bats are significant reservoir hosts for a wide array of pathogens. It should be underlined that they are main or occasional reservoirs of many viral (rabies, Marburg, Ebola, Hendra, Nipah, SARS-CoV, etc.), bacterial (*Bartonella*, *Leptospira*, *Mycoplasma*, *Brucella*, *Borrelia*, *Coxiella*, *Ehrlichia*, *Francisella*, *Mycobacteria* and *Rickettsia*), fungal (*Pseudogymnoascus destructans* - white-nose syndrome, *Histoplasma capsulatum* and others) and protozoan (malaria, toxoplasmosis, leishmaniasis, trypanosomiasis) infections. Direct transmission of pathogens from bats to humans is uncommon due to limited contact. However, pathogen spread often occurs through intermediaries like ectoparasites, bat guano, and other excretions. Humans can be affected either by the pathogens or the byproducts of their metabolism, such as toxins.

Climate changes directly or indirectly affect the distribution of pathogens in bat populations, increasing the risk of contact with humans and the likelihood of zoonotic disease transmission. The present review aims to describe the pathogenic viruses and bacteria identified in bats and their parasites, such as ticks, fleas, flies, and lice from the Northern temperate zone.

Human viral pathogens associated with bats

Bats are a suitable reservoir for viral zoonoses and more than 200 viruses have been isolated from or detected in bats (7,8). Bats are vectors

of about twenty viral families (9). Table 1 shows key viral families with zoonotic potential, including *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Hantaviridae*, *Herpesviridae*, *Paramyxoviridae*, *Parvoviridae*, *Poxviridae*, *Rhabdoviridae* and *Retroviridae*. These families comprise viruses that can be transmitted from animals to humans.

Different species of Chiroptera could be a reservoir of mutated viruses that extend the viral host range by overcoming the species barrier between bats and humans. This was suspected for SARS-CoV-2 that caused COVID-19 pandemics in 2020 (10). COVID-19 is a disease of presumed bat origin that emerged as a result of a mutation and was transmitted to humans from bats after overcoming the species barrier. The infection is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a member of the viral family *Coronaviridae*. Bats are reservoirs of this family worldwide and they are vectors of more members of *Coronaviridae* than any other taxa. It is suggested that the world pandemic started as a result of a mutation related to SARS-CoV-2 species in bats at the end of 2019 in the region of Wuhan, China. From January 2020 to April 28, 2024 the pandemic caused 7 047 396 registered deaths (<https://data.who.int/dashboards/covid19/deaths?n=c>) in the world, more than any infectious disease for the same period. A reservoir for SARS-CoV-2 and other similar viruses are *Rhinolophus* bats in Asia, which is one of the most common genera in Bulgaria, represented by 5 species. Possibly some mutations in bats or pangolins were the reason for overcoming the species barrier between bats and humans (11,12). Such evolution of viral fitness taking place in bats is possible and therefore investigation of bat-transmitted pathogens is very important.

Another widely spread viral family within the order Chiroptera is *Adenoviridae*. Members of this family are the cause of various diseases like conjunctivitis, gastroenteritis, and obesity (13). One of the bat vectors for *Adenoviridae* is *Pipistrellus kuhlii* spread in the South-Western and North-Eastern parts of Bulgaria. This bat species is synanthropic and its habitats are often buildings. *Pipistrellus kuhlii* and its excrements may pose an infection risk when in close contact with humans.

Table 1. Diseases and vectors of viral species identified in bats.

Viral family (representatives)	Bat host	Diseases	Secondary/ Intermediate vectors
<i>Coronaviridae</i>	<i>Rhinolophus</i>	COVID-19, MERS	pangolins, camels
<i>Adenoviridae</i>	<i>Pipistrellus kuhlii</i>	Respiratory Infections	n.a.
<i>Caliciviridae</i>	<i>Myotis daubentonii</i> <i>Eptesicus serotinus</i>	Gastroenteritis	vertebrates
<i>Flaviviridae</i>	Flying foxes	Ebola, Marburg	non-human primates
<i>Flaviviridae</i>	<i>Miniopterus schreibersii</i>	Japanese encephalitis virus (JEV)	ticks, mosquitos
<i>Herpesviridae</i> (Epstein-Barr virus)	<i>Myotis lucifugus</i>	Mononucleosis, Pneumonia. Kaposi's sarcoma, etc.	n.a.
<i>Poxviridae</i>	<i>Miniopterus schreibersii</i>	Smallpox	n.a.
<i>Retroviridae</i>	<i>Miniopterus</i> , <i>Rhinolophus</i>	AIDS	non-human primates
<i>Orthomyxoviridae</i> (H17N10 and H18N11)	New world bats	Influenza	birds
H9N2-like influenza A virus	Old world bats		

Bats together with cattle, pigs, cats, chickens, reptiles, dolphins, and amphibians are vectors of the viral family *Caliciviridae*. Members of caliciviruses are cause respiratory diseases, rabbit hemorrhagic disease (often with fatal hepatitis), and gastroenteritis (Norwalk group of viruses). *Myotis daubentonii* and *Eptesicus serotinus* are common carriers of caliciviruses and they are widely spread in Bulgaria.

Filoviridae is a viral family transmitting infections in humans and some animals. Two members of the family that are commonly known are the *Ebola virus* and the *Marburg virus*. Both viruses cause severe disease in humans and nonhuman primates in the form of viral hemorrhagic fevers (14). These two zoonoses have chiropteran vectors which are spread in Africa and are not present in our country.

Flaviviruses are a viral family whose transmission from animals to humans is mediated by arthropod vectors such as mosquitoes and ticks. They comprise the species *Zika virus*, and several other encephalitis viruses. One of the members of this family is a *Japanese encephalitis virus* (JEV) which is spread in Southeast Asia. One of the vectors of JEV - *Miniopterus schreibersii* - is a common and abundant species in Bulgaria, and in case of mutation, this disease might be transmitted to areas with a temperate climate (15).

Lyssaviruses are a genus of negative-sense single-strand RNA viruses in the family *Rhabdoviridae*. The genus *Lyssavirus* includes the rabies virus traditionally associated with the rabies disease. In Europe, bat *lyssaviruses* were detected in the United Kingdom, the Netherlands, Finland, Denmark, Poland, Czech Republic, Germany, Switzerland, France, Spain, Hungary, Italy, Slovenia, Croatia, Bulgaria, Ukraine, and Russia (7). Table 1 represents the distribution by viral family and bat species identified specifically in Northern temperate zone including Bulgaria for the last 16 years (9,16,17).

Betaherpesvirinae and *Gammaherpesvirinae* are subfamilies of *Herpesviridae* causing infections and certain diseases in animals and humans (18). Bats present in Bulgaria are common vectors of these two viral subfamilies (19). The widespread human cytomegalovirus (HCMV or HHV-5) is a Betaherpesvirus causing mucoepidermoid carcinoma. One of the gammaherpesviruses, *Epstein-Barr virus* (EBV or HHV-4), is implicated in several diseases, including mononucleosis and some cancers. Their vectors are European bat species present in Bulgaria (20).

Poxviridae is a viral family of double-stranded DNA viruses. The eradicated *Smallpox virus* belongs to this family. *Miniopterus schreibersii* is one of the vectors

of poxviruses (21).

The *Retroviridae* family of viruses includes the *Human immunodeficiency virus* (HIV), which is the etiological agent of acquired immunodeficiency syndrome (AIDS) and other zoonotic diseases. *Miniopterus* and *Rhinolophus* bats are common vectors of deltaretroviruses (22).

Human bacterial pathogens associated with bats

Most of the human bacterial pathogens associated with bats are food-borne related bacterial pathogens, zoonoses like brucellosis, tularemia (23), and atypical mycobacterial infections. Bats can spread the bacteria during their migration directly or mediated by their ectoparasites like mosquitos, ticks, fleas, and lice. Many of these pathogenic bacteria belong to Risk Group 2 or higher according to the guidelines of the National Institutes of Health (NIH) (<https://absa.org/riskgroups/>).

The most common bacterial pathogenic family observed in the gut of bats is Enterobacteriaceae. This family includes mainly food-borne pathogens, 1–5 µm long, Gram-negative rods (24). Many representatives of Enterobacteriaceae are part of the normal intestinal microflora and can be found in the intestines of humans and other animals, while the rest live in soil, water, or are parasitize various plants and animals. Seventeen human pathogenic genera of Enterobacteria (*Enterobacter*, *Citrobacter*, *Hafnia*, *Serratia*, *Providencia*, *Klebsiella*, *Morganella*, *Escherichia*, *Proteus*, *Cronobacter*, *Kluyvera*, *Moellerella*, *Leclercia*, *Pantoea*, *Rahnella*, *Salmonella*, and *Shigella*) were identified in bats across Europe. *Myotis myotis*, *Miniopterus schreibersii*, *Rhinolophus hipposideros*, and *Myotis capaccinii* are common vectors of Enterobacteria species (25). *Yersinia pseudotuberculosis*, an enteric human pathogen, has been observed to cause outbreaks in European bat populations. Another enteric human pathogen – *Yersinia pseudotuberculosis* is not typically a pathogen of bats, but it can cause outbreaks in European bat populations (24,26). Table 2 displays the range of microbial pathogens that are present in bats.

Leptospira species like *L. interrogans* and *L. kirschneri* are pathogens that affect both humans and bats. These bacteria infect the renal tubules of animals

and people and are excreted via urine into the environment. Most bat vectors of *Leptospira* are spread in tropical regions but recent studies showed the presence of this bacterium in *Barbastella barbastellus*, *Myotis bechsteinii*, *Myotis myotis*, and *Myotis nattereri* (27). Dogs and rodents are also carriers of this pathogen. (28,29).

Tularemia is a disease caused by *Francisella tularensis* considered a high-risk agent with the potential to be used as a biological weapon. Recently this bacterium has been identified in the bats *Pipistrellus pipistrellus*, *Nyctalus noctule*, *Pipistrellus kuhlii*, and their ectoparasites (22). Other bacterial agents belonging to special pathogens are the species of the genus *Brucella*, the causative agent of brucellosis. *Brucella* species were identified in *Myotis blythii* (30). *Bartonella* is a genus of Gram-negative facultative intracellular parasitic bacteria. *Bartonella* species are transmitted by ticks, fleas, and mosquitoes. These bacteria are responsible for bartonellosis (Carrión's disease, trench fever, cat-scratch disease, bacillary angiomatosis, peliosis hepatis, chronic bacteremia, endocarditis, chronic lymphadenopathy, and neurological disorders). Bat species *M. schreibersii* and *M. blythii* are vectors of *Bartonella* species (26). *Pasteurella* is a genus of Gram-negative, facultative anaerobic bacteria causing pasteurellosis, a skin or subcutaneous tissue disease like septic phlegmon which develops most often on hand or forearm after a cat bite. After infection, edema, severe pain, and serosanguineous exudate appear very rapidly. Transmission of *Pasteurella* from bats to humans is not proven, but the presence of these bacteria is common in chiropteran species like *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus*, *Pipistrellus kuhlii*, *Plecotus auratus*, *Vespertilio murinus*, *Pipistrellus nathusii*, *Myotis mystacinus*, *Vespertilio murinus*, *Plecotus auritus*, and *Eptesicus serotinus* (27).

Nontuberculous Mycobacteria (NTM) are emerging pathogens causing opportunistic infections in humans and animals. Their distribution in caves and bats in Bulgaria is poorly studied. A recent study performed by Atanasova et al. (31) demonstrated that most of the NTMs were identified in bat guano (67%, n=16). Other materials positive for NTMs were water and biofilm (13%, n=3 each), sediment and clay (3%, n=1 each). The mycobacterial species diversity identified in the

Table 2. Human bacterial pathogens found in European bats.

Microbial taxa	Carriers	Diseases	Alternative vectors
<i>Brucella</i> spp.	<i>Miniopterus schreibersii</i> , <i>Myotis blythii</i>	Brucellosis	Domestic animals
<i>Francisella tularensis</i>	<i>P. pipistrellus</i> , <i>Nictalus noctula</i> , <i>P. kuhlii</i>	Tularemia	Arthropods, mammals
Enterobacteriaceae	Chiroptera order	Gut infections	
<i>Yersinia pseudotuberculosis</i>	<i>Myotis myotis</i>	Far East scarlet-like fever	Animals and birds
<i>Leptospira</i> spp.	<i>Barbastella barbastellus</i> , <i>Myotis bechsteinii</i> , <i>Myotis myotis</i> , <i>Myotis nattereri</i>	Renal infections	Dogs
<i>Pasteurella</i> (<i>P. multocida</i> , <i>Pasteurella canis</i> , <i>Pasteurella dagmatis</i> , <i>Pasteurella stomatis</i>)	<i>Pipistrellus pipistrellus</i> , <i>Pipistrellus pygmaeus</i> , <i>Pipistrellus kuhlii</i> , <i>Plecotus auratus</i> , <i>Vespertilio murinus</i> , <i>Pipistrellus nathusii</i> , <i>Myotis mystacinus</i> , <i>Vespertilio murinus</i> , <i>Plecotus auritus</i> , and <i>Eptesicus serotinus</i>	Pasteurellosis	Cats, dogs
<i>Bartonella</i> (<i>B. bacilliformis</i> , <i>B. quintana</i> , <i>B. henselae</i>)	<i>M. schreibersii</i> and <i>M. blythii</i>	Bartonellosis Carrión's disease, trench fever, cat-scratch disease, bacillary angiomatosis, peliosis hepatis, chronic bacteremia, endocarditis, chronic lymphadenopathy, and neurological disorders	Cats, dogs, rodents

samples included: *M. chelonae* (n=3), *M. gordonae* (n=2), *M. intermedium* (n=3), *M. scrofulaceum* (n=1), *M. szulgai* (n=4), *M. fortuitum* group (n=4), mixed culture (n=4), *M. terrae* complex (n=1). Similar results were obtained by Pavlik et al. (32) by studying a total of 281 guano samples collected from caves (N = 181) in eight European countries (Bulgaria, Czech Republic, France, Hungary, Italy, Romania, Slovakia and Slovenia). The authors reported that 73 mycobacterial isolates were identified as members of three groups (*M. fortuitum*, *M. chelonae*, and *M. mucogenicum*) and four complexes (*M. avium*, *M. terrae*, *M. vaccae*, and *M. smegmatis*). A total of 20 isolates (22.5%) belonged to risk group 1 (environmental saprophytes), 48 isolates (53.9%) belonged to risk group 2 (potential pathogens), and none of the isolates belonged to risk group 3 (obligatory pathogens). The presence of NTM in cave ecosystems represents a potential source for human infection.

Recently our group has studied the blood microbiome of bats. Our results showed that a significant number

of non-pathogenic and pathogenic bacterial species could be detected in bats blood. The study included shotgun metagenomics sequencing combined with a bioinformatics approach for microbial identification. Sequencing reads exceeding 10K were identified for *Mycobacteria*, *Bartonella*, *Plasmodium* and *Leishmania* species. Soil, water, and guano samples demonstrated high abundancy of mycobacterial presence. Other pathogenic species such as *Toxoplasma* spp. have also been identified but less evidently according to the number of reads. Our results demonstrated that bats in Bulgaria are a hidden or potential reservoir for malaria and atypical mycobacterial infections. Mycobacterial presence was confirmed by targeted conventional PCR.

Fungal infections in bats

Bats can potentially be carriers and spreaders of fungal infections (33). Fungal infections have been identified as either contracted by bats themselves or as bat-borne mycoses in other host species, including

humans. Research data on the fungal microflora present in the gut of different bat species and their dietary habits is limited. Reports on the occurrence of fungal species in the bats' closer environment, such as ectoparasites or bat guano, are rare. The most well-known association between bats and mycotic disease in human patients is *Histoplasma capsulatum*. Infections caused by this fungus are contracted while visiting caves inhabited by bats in tropical countries.

The distribution and impact of white-nose syndrome, caused by the psychrophilic fungus *Pseudogymnoascus destructans* (formerly *Geomyces destructans*), on European bats has yet to be fully determined (34). Dermal fungal infiltrations were observed in biopsies of bats belonging to *Myotis blythii*, *M. daubentonii*, *M. emarginatus*, and *M. myotis*. of A higher susceptibility to infection of the *Myotis* genus, especially *M. emarginatus* was suggested, possibly due to the longer hibernation period of these bats. Genomic studies indicate that *P. destructans* is native to Eurasia (35). No human infections attributable to *Pseudogymnoascus destructans* have been reported. Studies have shown that the fungus grows at temperatures much lower than that of the human body. Temperature fluctuations during bat's flight and rest may pose a risk for breaching the species barrier between bats and humans.

Some causative agents of parasitoses are distributed by European bats, especially in pair with their arthropod ectoparasites like ticks and flies (36). Intracellular parasitic eukaryotic Apicomplexa with the main human pathogenetic species *Toxoplasma gondii* is spread by *M. schreibersii*. Most bat species in Bulgaria carry ectoparasitic insects that can transmit parasites from the genus *Polychromophilus*, phylogenetically closely related to the parasite that causes malaria in humans – *Plasmodium falciparum* and other plasmodia.

CONCLUSIONS

Bats are the second largest order of mammals and a significant reservoir and carrier of viral, bacterial, and fungal pathogens, some of which are particularly dangerous. The role of bats in the incidence and mutagenesis of potential pathogens and potential overcoming of the species barrier is related to the

ability to fly over long distances, their longevity, colony structure, coexistence with different species including humans, and the fluctuations of their metabolism and body temperature. Investigation of the Chiropteran microbiome is very important for human health and the potential prevention of future epidemics. Currently, there are no comprehensive studies characterizing the metagenomic diversity of bats, the related blood-sucking ectoparasites, and the interaction with their surrounding environments, including caves, water, and soil. Understanding the underlying mechanisms of those process is essential for the development of strategies for mitigating the risks associated with zoonotic diseases. Combating new and emerging infections represents a significant global issue and societal challenge.

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