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Bacterial Communities of Bartonella-Positive Fleas in Gut Microbiota of Armenian Populations

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ABSTRACT

Bartonella spp. are known as causative agents of zoonosis. The information on the reservoirs of *Bartonella spp.* mammals /fleas/ ticks, is limited in Armenia. The aim of this study was, on the basis of the available PhyloChip™ data from the previous investigations, to study bacterial communities in healthy and patients with Familial Mediterranean Fever of the Armenian population that have common gene sequences with *Bartonella*-positive fleas.

The preliminary results on PhyloChip™ analysis revealed operational taxonomic units of several gut bacterial communities in healthy people and patients with FMF in the Armenian population sharing common gene-sequences with the *Bartonella*- positive fleas.

Introduction

Bartonella spp. bacteria can be observed around the globe and are the causative agents of emerging and reemerging human diseases. Bacteria of the genus *Bartonella* are fastidious, gradually-growing gram-negative aerobic rods. They parasitize erythrocytes and endothelial cells of a wide range of mammals and are generally host specific at different taxonomical levels (Bai, et al., 2013, Breitschwerdt, 2017, Kosoy, 2018, Ying, et al., 2002) (Table 1).

Rapid growth of populations, particularly in areas with weak health systems, urbanization, globalization and inequalities within cities, climate change, and the changing nature of pathogen transmission between

human and animal populations are an important cause of zoonosis. *Bartonella* species, widely known as causative agents of zoonosis, involve a broad spectrum of clinical syndromes from self-limited cat-scratch disease (CSD) to potentially fatal diseases, such as endocarditis (Chomel, 2009) mild lymphadenopathy and fever (Anderson, et al., 1997, Kosoy, et al., 2010, 2018, Chomel, et al., 2004), and their DNA can be found in multiple vectors (Cheslock and Embers, 2019).

Pets (Chomel, et al., 2006), especially cats (Razgūnait, et al., 2021), are large reservoirs for human infection (Angelakis, et al., 2014, Iannino, et al., 2018). The disease is widely transmitted to cats (Petříková, et al., 2021) and

rodents (Gutiérrez, et al., 2015) by fleas. It has also been discovered in a wide range of mammals, including humans, cats, dogs, rabbits, rodents, horses, cattle, and other wildlife (Chomel, et al., 2009, Iannino, et al., 2018, Breitschwerdt, et al., 2017).

Materials and methods

Historically, the most common causative agents for human cases of bartonellosis have been *Bartonella bacilliformis*, *Bartonella quintana*, and *Bartonella henselae* (Angelakis, et al., 2014, Breitschwerdt, et al., 2019). These infections are characterized by a prolonged intraerythrocytic bacteremia, fever, headache, and malaise to more severe

symptoms such as hallucinations (Ben-Tekaya, et al., 2013, Kalogeropoulos, et al., 2019).

According to retrieved PubMed information, several cases of bartonellosis have been reported in Caucasus (Malania, et al., 2016), but the information on the reservoirs of *Bartonella spp.*: mammals/fleas/ticks, is limited in Armenia. The third generation, culture-independent, high-density DNA microarray (PhyloChip™; Affymetrix, Santa Clara, CA, USA) (Harutyunyan, et al., 2013, 2014a, 2014b, Pepoyan, et al., 2014a, 2014b, 2015a, 2015b, 2018, 2019, 2021, Piceno, et al., 2013) was used during our previous investigations on gut microbiota of Familial Mediterranean Fever (FMF) patients. The aim of this study was, on the basis of the available PhyloChip™ data from the previous investigations, to study bacterial communities in healthy people and patients with FMF in the Armenian population that have common gene sequences with *Bartonella*-positive fleas.

Table 1. The known *Bartonella* species, their hosts and their vectors*

Bartonella Species	Host (s)	Vector(s)
<i>B. henselae</i>	Cat, human, dogs, horses	Fleas, lice, ticks, spiders
<i>B. quintana</i>	Humans, macaques, cats, dogs	Human body lice, fleas, bed bugs
<i>B. bacilliformis</i>	Humans	Sandflies, fleas
<i>B. koehlerae</i>	Cats, dogs, humans	Fleas
<i>B. vinsonii ssp. berkhoffi</i>	Dogs, horses, foxes, humans	Fleas, ticks
<i>B. bovis</i>	Cattle, cats, dogs, human	Biting flies, ticks
<i>B. clarridgeiae</i>	Cats, dogs	Fleas, ticks
<i>B. rattimassiliensis</i>	Rats	Fleas
<i>B. tamiae</i>	Rats, humans	Mites
<i>B. tribocorum</i>	Rats	Fleas
<i>B. rousetii</i>	Bats	Bat flies
<i>B. schoenbuchensis</i>	Cattle	Biting flies, ticks
<i>B. chomelii</i>	Cattle	Biting flies, ticks
<i>B. doshiae</i>	Rats, humans	Fleas
<i>B. grahamii</i>	Mice, humans	Fleas
<i>B. birtlesii</i>	Mice	Fleas
<i>B. mayotimonensis</i>	Bats, humans	Bat flies, fleas, ticks
<i>B. elizabethae</i>	Rats, humans, dogs	Fleas
<i>B. washoensis</i>	Dogs, humans	Fleas, ticks
<i>B. rochalimae</i>	Dogs, humans	Fleas, ticks
<i>B. vinsonii ssp. arupensis</i>	Dogs, humans	Fleas, ticks
<i>B. melophagi</i>	Sheep, humans	Sheep keds
<i>B. alsatica</i>	Rabbits, humans	Fleas, ticks

*The table has been adapted from Breitschwerdt E.B., 2017, Chapter 5.2.

Results and discussions

Forty healthy and forty FMF volunteers participated in investigations. A detailed information of these investigations is available at Pepoyan et al. (Pepoyan, et al., 2018, www.ncbi.nlm.nih.gov).

Also, the literature data were collected from PubMed (1997-2021, main keywords: *Bartonella spp* and Caucasus).

Bacterial communities of *Bartonella*-positive fleas in gut microbiota of Armenian healthy and FMF patients' populations are presented in Table 2. The pilot PhyloChip™ analysis revealed operational taxonomic units (OTU) of several gut bacterial communities in healthy people and patients with FMF in the Armenian population sharing common gene-sequences with the *Bartonella*-positive fleas. According to Table 2, *Proteobacteria*, *Fusobacteria* and *Firmicutes* were the main bacterial phyla sharing common gene-sequences with the *Bartonella*-positive fleas. There were no statistically significant differences in the hybridization scores of these bacteria from the healthy and diseased groups.

The investigations on the seroprevalence against *B. henselae* and *B. quintana* of 536 people from the Eastern Slovakia, with no data on the human bartonellosis cases as it was in Armenia, revealed that 23.5 % of the people demonstrated positivity for anti-*B. henselae* antibodies and 24.8 % against *B. quintana* (Petříková, et al., 2021). Statistically significant, but clinically irrelevant differences in uric acid levels and serum creatinine were described between *B. henselae* seropositive and seronegative groups (Petříková, et al., 2021).

Table 2. Bacterial communities of *Bartonella*-positive fleas in gut microbiota of Armenian healthy and FMF patients' populations*

Bacterial genus	Healthy Men volunteers (N=40)	Male FMF volunteers (N=40) (hybridization score)
<i>Haemophilus</i>	598±105 ^a	432 ± 125 ^a P>0.05
<i>Aquabacterium</i>	4137±760 ^a	5005 ± 1351 ^a P>0.05
<i>Aquabacterium</i>	1408±507 ^a	1970 ± 102 ^a P>0.05
<i>Aquabacterium</i>	3070±885 ^a	3673 ± 189 ^a P>0.05
<i>Faecalibacterium</i>	9642±257 ^a	9268 ± 251 ^a P>0.05
<i>Oscillospira</i>	1930±922 ^a	2623 ± 119 ^a P>0.05

N – Number of volunteers
a-hybridization score
P < 0.05 is statistically significant

*Composed by the authors.

Conclusion

Thus, future investigations on reservoirs of *Bartonella* spp. in mammals/fleas/ticks in Armenia, as well as future immunologic and molecular biological investigations on transmission of *Bartonella* spp. are required.

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