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BRIEF REPORT

First detection of *Bartonella* spp. in bat bugs *Cimex pipistrelli* (Hemiptera: Cimicidae), Central Europe

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Abstract

Bats are an important reservoir for many viral pathogens in humans. However, their role in the transmission of bacterial pathogens is neglected, as is that of their ectoparasites. This study focuses on the molecular detection of *Bartonella* spp. in bat bugs *Cimex pipistrelli* using partial sequences of *gltA* (citrate synthase), *ssrA* (transfer messenger RNA, *tmRNA*), and the 16S-23S rDNA internal transcribed spacer (ITS) region as targets. *Bartonella* DNA was detected in 2/112 (1.79% prevalence) samples from bat bugs. Due to the fact that bat bugs can sporadically bite humans, more extensive surveillance and vector competence studies are needed to ascertain zoonotic risk of bat-associated *Bartonella* spp.

Keywords *Bartonella* spp. · Bat · *Cimex pipistrelli* · Bat bug

Introduction

Bats are considered important reservoirs and vectors of various pathogens, especially of viruses (Calisher et al. 2006). Recent studies show that bats may also harbor a variety of bacterial pathogens (e.g., *Leptospira*, *Bartonella*, *Yersinia*, *Rickettsia*, and *Escherichia* spp.), but the prevalence of these pathogens in bats and their ectoparasites, modes of transmission, zoonotic potential, and public health implications remain poorly understood (Mühldorfer 2013; Stuckey et al. 2017).

Bats can be infested by a variety of ectoparasites. Some of them such as bat flies (Diptera: Nycteribiidae, Streblidae), bat mites (Acari: Spinturnicidae, Macronyssidae), and bat fleas (Siphonaptera: Ischnopsyllidae) are highly host specialized, making it unlikely that they feed on other mammalian species or even humans, and their potential role in *Bartonella* transmission is between bat individuals or between different bat species (Hornok et al. 2012; Morse et al. 2012; Bendjeddou et al. 2017). In contrast, less host-associated ectoparasites such as ticks and cimicids are known to occasionally bite humans and therefore pose a risk for potential direct *Bartonella* transmission to humans. Infestation of humans by bat ticks *Argas vespertilionis*, *Ornithodoros hasei*, or *Ixodes vespertilionis*, which are known to harbor bat-associated *Bartonella*, has been documented in Sweden, *French Guiana*, and Poland, respectively (Jaenson et al. 1994; Davoust et al. 2016; Piksa et al. 2013; Hornok et al. 2019). Invasion of *A. vespertilionis* ticks into the attic of houses has also been reported, including various pathogenic bacteria detected in the collected ticks (Socolovschi et al. 2012). To our knowledge, the only case report of humans exposed to the bat bug *Cimex pipistrelli* is from the UK. In this case, people were infested by bat bugs that originated from a large bat colony that resided in the room above the ceiling. The bat bugs had access through gaps in the floor and through pipes in the bathroom. Human contact with bat bugs is thus quite rare, but possible (Whyte et al. 2001).

Romana Kejíková and Clifton McKee contributed equally to this work.

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Bat bugs *Cimex pipistrelli* are common ectoparasites in bat roosts, typically residing on the host's skin only during a blood meal (Bartonička and Růžičková 2012). Our study aims to determine the presence of bat-associated *Bartonella* spp. in bat bugs *Cimex pipistrelli*. To date, few studies have attempted detection of bartonellae in bat bugs: in *Cimex adjunctus* in the USA and Canada (Reeves et al. 2005; Lilley et al. 2017; McKee et al. 2018), in *Cimex pipistrelli* in the Netherlands (McKee et al. 2019), and in *Cimex japonicus* in Japan (Nabeshima et al. 2022). Given this limited knowledge, it is necessary to assess the prevalence and distribution of bartonellae in bat bugs and to evaluate their zoonotic potential with respect to possible human contact with bats (Whyte et al. 2001).

Material and methods

Sampling and DNA isolation

A total of 112 individual bat bugs were sampled in nursery colony of *Myotis myotis* (June 2018) in Jevišovice, SW Moravia, Czech Republic. Samples were transported to the laboratory in cooled containers. Upon arrival, the samples were stored at −60 °C until further processing. All bat bugs were determined to be *Cimex pipistrelli* (Usinger 1966). Bat bugs were surface-sterilized using a 70% ethanol (5 min) and then rinsed in sterile 1x PBS (Oxoid, England). Bugs were homogenized individually in 100 µl PBS (Oxoid, England) using the automated homogenizer TissueLyser II (Qiagen/Retsch, Hilden, Germany). DNA was extracted using the QiaAMP DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Conventional PCR

For initial screening, *Bartonella*-specific conventional PCR was performed targeting 16S-23S rDNA internal transcribed spacer (ITS) region with primers BA325s (5′-CTTCAGATG ATGATCCCAAGCCTTCTGGCG-3′) and BA1100as (5′-GAACCGACGACCCCCTGCTTGCAAAGCA-3′) (Maggi et al. 2006). In case of positivity, additional loci were targeted by conventional PCR as described (Bai et al. 2013)═firstly, the transfer-messenger RNA region with *ssrA* primers (5′-GCT ATGGTAATAAATGGACAATGAAATAA-3′) and (5′-GCT TCTGTTGCCAGGTG-3′) (Diaz et al. 2012); secondly, a partial sequence of citrate synthase gene (*gltA*) with primers BhCS 781p (5′-GGGGACCAGCTCATGGTGG-3′) and BhCS 1137n (5′-AATGCAAAAAGAACAGTAAACA-3′) (Norman et al. 1995). Each reaction tube contained 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.001% Tween 20, 2.5 mM $MgCl_2$, 200 mM mixture of dNTPs, 2.5 U Taq purple DNA polymerase (Top-Bio, Prague, Czech Republic), and 25 pmol of each primer. PCR was performed using an Eppendorf Mastercycler 5341 (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation 5 min at 94 °C, followed by 40 cycles of 30 s denaturation at 94 °C, 30 s annealing at 66 °C and 50 s extension at 72 °C, and final extension of 5 min at 72 °C. Amplified products were separated using gel electrophoresis with 1.5% agarose gel stained with GelRed® (Biotium, USA) and visualized under UV-light. The processing of mosquito pools, DNA extraction, PCR setup (preparation of mastermix, addition of primers, PCR reaction), and post-PCR procedures (agarose gel electrophoresis) was carried out in separate rooms to avoid cross-contamination.

Sequence analysis of PCR products

PCR products were purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, USA); amplification was performed using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 1.1 (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions, and then purified with EtOH/EDTA precipitation. Bidirectional sequencing was performed using ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA). The obtained DNA sequences were processed and aligned using the Seqman module in Lasergene v12 (DNASTAR Inc., USA) and manually checked. The algorithm BLAST (http://www.ncbi.nlm.nih.gov/blast) from the National Center for Biotechnology Information (Bethesda, MD, USA) was used for database screening.

Phylogenetic analysis

Starting with a compiled database of *Bartonella* sequences of multiple genetic loci (including *gltA*, ITS, *ssrA*) from named *Bartonella* species and strains identified in bats, we added reference sequences from GenBank that closely matched after a BLAST search and additional *Bartonella* sequences previously amplified from bat bugs for comparison (McKee et al. 2021). Alignments for each gene were made with MAFFT v7 using the L-INS-i algorithm (Katoh and Standley 2013). Alignments for ITS and *ssrA* were trimmed manually because they are not protein-coding and possess insertions and deletions in the aligned sequences while the alignment for *gltA* was trimmed using Gblocks v0.91b (Castresana 2000) because it is protein-coding and contained no gaps. Simultaneous sequence evolution model selection and phylogenetic tree inference were performed with IQTREE v2.1.1 (Minh et al. 2020).

Results and discussion

A total of 112 individuals of *Cimex pipistrelli* bats were processed individually and examined for the presence of *Bartonella* spp. Two samples (designated as C24 and C79

Table 1 Comparison on *Bartonella* sequences amplified from *Cimex pipistrelli* with other sequences on GenBank via BLAST query

Sequence	Closest ITS matches on GenBank	Closest *ssrA* matches on GenBank	Closest *gltA* matches on GenBank
C24	(1) *Bartonella* sp. Iv76, *Ixodes vespertilionis*, Romania (MH544203) – 100% (304/304) (2) *Bartonella* sp. 44591, *Myotis blythii*, Georgia (MF288124) – 95.2% (318/334)	(1) *Bartonella* sp. B41044, *Phyllostomus discolor*, Guatemala (MN529288) – 90.7% (274/302) (2) *Bartonella* sp. B32728, *Macrophyllum macrophyllum*, Guatemala (MN529309) – 90.7% (274/302)	No sequence
C79	(1) *Bartonella* sp. clone #68, *Myotis blythii*, Georgia (KX420737) – 100% (434/434)	(1) *Bartonella* sp. GU-21 B43023, *Pteronotus davyi*, Guatemala (MN276374) – 93.3% (279/299) (2) *B. bovis*, *Bos taurus*, Malaysia (KR733196) – 93.1% (268/288)	(1) *Bartonella* sp. SD-124, *Myotis pequinius*, China (KX655819) – 95.5% (322/337) (2) *Bartonella* sp. SD-64, *Myotis fimbriatus*, China (KX655813) – 95% (360/379)

from *M. myotis*) were positive for *Bartonella* spp. sequences from the ITS region (deposited in GenBank under accession numbers OM501582 and OM501583); *ssrA* sequences were obtained from both samples (deposited in GenBank under accession numbers OM514677 and OM514678); and a single *gltA* sequence was amplified from C79 (deposited in GenBank under accession number OM514679) (Table 1). Both ITS sequences shared high sequence identity (> 95%) with *Bartonella* sequences detected in *Myotis* spp. bats from Georgia (Bai et al. 2017) and sample C24 shared 100% sequence identity with an ITS sequence in a bat tick (*Ixodes vespertilionis*) collected in Romania (Hornok et al. 2019) (Supplementary Fig. 1A). The two *ssrA* sequences did not have any matches on GenBank with high (> 95%) sequence identity due to the infrequent use of this marker for genotyping, but the closest matches were to *ssrA* sequences from phyllostomid bats sampled in Guatemala (Bai et al. 2011; McKee et al. 2021) and with *Bartonella* species found in ungulates (e.g., *B. bovis*, *B. melophagi*, *B. schoenbuchensis*) (Supplementary Fig. 1B). The single *gltA* sequence from sample C79 most closely matched sequences previously detected in *Myotis* spp. bats from China (Han et al. 2017) (Table 1; Supplementary Fig. 1C). Compared to ITS and *gltA* sequences previously detected in bat bugs (Reeves et al. 2005; Lilley et al. 2017; McKee et al. 2018, 2019), the new sequences from *C. pipistrelli* described here are not closely related (Supplementary Fig. 1A and C).

Bat-associated bartonellae are increasingly recognized bacteria among a wide range of bat species and their ectoparasites. To date, numerous studies have been published from several countries around the world, most of them from Africa, South America, and Asia with a focus on frugivorous and insectivorous bat species from tropical and subtropical regions. In particular, bartonellae have been detected in bat samples and bat ectoparasites from Kenya, Nigeria, Madagascar, South Africa, Taiwan, Thailand, China, Costa Rica, Peru, Mexico, Brazil, and Argentina (Stuckey et al. 2017).

Several studies have been published on the detection of *Bartonella* spp. in bats and their ectoparasites in mild northern hemisphere climates, but the zoonotic potential of these bartonellae remains unclear (Concannon et al. 2005; Stuckey et al. 2017; Corduneanu et al. 2018; Sándor et al. 2018; Szubert-Kruszyńska et al. 2019). Interestingly, Veikkolainen et al. (2014) detected *Bartonella* sequences in Finnish bats that were closely related to "*Candidatus* Bartonella mayotimonensis," which was originally isolated from the aortic valve of a patient with endocarditis in Iowa (Lin et al. 2010). Later, Lilley et al. (2017) confirmed sequences identical to "*Candidatus* Bartonella mayotimonensis" in American bats. In addition, Urushadze et al. (2017) identified *Bartonella* strains in Georgian bats closely related to sequences detected in forestry workers in Poland (Podsiadły et al. 2011). Although the zoonotic potential of bat-associated *Bartonella* remains undetermined, the risk of possible transmission and spillover from bat to human through contact with bats or their ectoparasites should be considered. However, the source of infection in humans remains unknown (Veikkolainen et al. 2014; Lilley et al. 2017; Urushadze et al. 2017).

Further studies such as those by Mannerings et al. (2016) and Bai et al. (2018) in Ghana and Nigeria, respectively, are needed to assess human exposure to bat-associated bartonellae, with a focus on public health risk because *Bartonella* infection is often the causative agent of severe culture-negative endocarditis (Okaro et al. 2017).

Our study shows for the first time the presence of novel *Bartonella* spp. in bat bugs *Cimex pipistrelli* in Central Europe. Bat roosts are often infested with bat bugs and people living near roosting bats should be aware of contact with bats and their bugs. This study provides additional knowledge about pathogens in bat ectoparasites. The bacterial flora of European bats needs to be studied in more detail, as there is a potential risk of transmission of some bacterial species to humans, other animals, pets, or livestock.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-022-07668-4.

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Author contribution IR, CM, and RK designed the study. RK and PS carried out basic molecular analysis, JM performed the sequence analysis, and CM designed the phylograms and performed the corresponding data interpretation. RK, IR, SŠ, and CM drafted the manuscript. All authors read and approved the final manuscript version.

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Data availability Data supporting the conclusions of this article are included within the article. Representative DNA sequences have been deposited in the GenBank database under the accession numbers (OM501582; OM501583; OM514677; OM514678; OM514679). All the sequences and their accession numbers included in the phylogenetic analysis are provided as supplementary data.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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