Prevalence, diversity, and host associations of *Bartonella* strains in bats from Georgia (Caucasus)

Lela Urushadze¹,²*, Ying Bai³, Lynn Osikowicz³, Clifton McKee³,⁴, Ketevan Sidamonidze¹, Davit Putkaradze¹, Paata Imnadze¹, Andrei Kandaurov⁵, Ivan Kuzmin⁶, Michael Kosoy³

¹ National Center for Disease Control and Public Health, Tbilisi, Georgia, ² Ilia State University, Tbilisi, Georgia, ³ Centers for Disease Control and Prevention, Division of Vector-Borne Disease, Fort Collins, Colorado, United States of America, ⁴ Department of Biology, Colorado State University, Fort Collins, Colorado, United States of America, ⁵ Institute of Zoology, Ilia State University, Tbilisi, Georgia, ⁶ Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America

* lelincdc@gmail.com

Abstract

*Bartonella* infections were investigated in seven species of bats from four regions of the Republic of Georgia. Of the 236 bats that were captured, 212 (90%) specimens were tested for *Bartonella* infection. Colonies identified as *Bartonella* were isolated from 105 (49.5%) of 212 bats. Phylogenetic analysis based on sequence variation of the *gltA* gene differentiated 22 unique *Bartonella* genogroups. Genetic distances between these diverse genogroups were at the level of those observed between different *Bartonella* species described previously. Twenty-one reference strains from 19 representative genogroups were characterized using four additional genetic markers. Host specificity to bat genera or families was reported for several *Bartonella* genogroups. Some *Bartonella* genotypes found in bats were clustered with those identified in dogs from Thailand and humans from Poland.

Author summary

Bacteria of the genus *Bartonella* parasitize erythrocytes and endothelial cells of a wide range of mammals and recently were reported in bats from Africa, Asia, America, and northern Europe. A human disease case in the USA was associated with a novel *Bartonella* species, which later was identified in bats in Finland. This human case has demonstrated the zoonotic potential of bat-borne *Bartonella* and underscores the need for extended surveillance and studies of these pathogens. The present work assesses prevalence and diversity of *Bartonella* in bats in the country of Georgia (southern Caucasus), characterizes reference strains representing diverse genogroups by variation of genetic loci, and evaluates the links between identified *Bartonella* genogroups and bat hosts. Importantly, some *Bartonella* genotypes found in bats were close or identical to those identified in dogs and humans. The data indicate that the public health impact of *Bartonella* carried by bats should be investigated.
Introduction

Bats (Order: Chiroptera) are hosts of a wide range of zoonotic pathogens. Their significance as reservoirs of emerging infectious diseases, predominantly of viral origin, has been increasingly recognized during recent decades [1,2]. In contrast, the study of bacterial infections in bats has progressed more slowly [3]. Bacteria of the genus Bartonella are small and slow-growing Gram-negative aerobic bacilli. These bacteria parasitize erythrocytes and endothelial cells of a wide range of mammals. During the last six years, diverse Bartonella strains were identified in bats from Europe [4–6], Africa [7–12], Asia [13,14], and Latin America [15–19]. Recent studies have demonstrated significant patterns of evolutionary codivergence among bats and Bartonella, demonstrating that strains of Bartonella in bats tend to cluster according to bat families, superfamilies, and suborders [20,21]. Host specificity and codivergence have also been documented in rodent-associated Bartonella strains [20,22] and bat-associated Leptospira strains [23]. Despite their apparent host associations, Bartonella spp. can spillover into phylogenetically distant hosts, including humans [24,25]. A recent human case of endocarditis in the US Midwest was associated with a novel Bartonella species (B. mayotimonensis; [26]), which later was isolated in bats in Europe [5]. This human case has demonstrated the zoonotic potential of bat-borne Bartonella and underscores the need for extended surveillance and studies of these pathogens.

The goal of the present work was to identify prevalence and diversity of Bartonella in bats in the Republic of Georgia (southern Caucasus) with the following objectives: 1) to compare prevalence of Bartonella infection in diverse bat species from different geographic locations within Georgia; 2) to determine the genotypes of obtained strains by variation in gltA sequences, a gene commonly used for discrimination of Bartonella species; 3) to characterize reference strains representing diverse genogroups by variation of multiple genetic loci; and 4) to evaluate the links between identified Bartonella genogroups and bat hosts.

Materials and methods

Ethics statement

All animal work has been conducted according to relevant NCDC, national, and international guidelines.

Capture and sample collection

Bats were collected from two distinct parts of Georgia in June 2012. Four locations are situated in Eastern Georgia: three sites in the Kakheti region near Davit Gareja, one site in the Kvemo Kartli region in Gardabani district. The other four locations are in Western Georgia: two sites in the Samegrelo-Zemo Svaneti region (Martvili district and Chkhrotsku district) and two sites in the Imereti region (Terjola district and near Tskaltubo town). The number of captured bats from each site is shown in Table 1.

Bats were captured manually or using nets from different roosts in caves and buildings (attics, cellars, and monasteries). The list of bat species and the number of animals per roost or colony available for sampling was approved by the Ministry of Environmental and Natural Resources Protection of Georgia. Species of captured bats were identified based on external morphological characteristics. Captured bats (n = 236) were delivered to the processing site in individual cotton bags where they were processed. Bats were anesthetized with the use of ketamine (0.05–0.1 mg/g body mass) and exsanguinated by cardiac puncture. All bats were sexed and measured. The procedures of handling animals were performed in compliance with the protocol approved by the CDC Institutional Animal Care and Use Committee (protocol 2096FRAMULX-A3). Blood specimens were transported on dry ice to the NCDC Laboratory,
Tbilisi where they were stored at -80˚C until they were shipped on dry ice to the CDC’s laboratory, Fort Collins, Colorado. Upon arrival at CDC, the samples were stored at -80˚C until they were analyzed.

### Culturing

Bat blood was diluted 1:4 in Brain Heart Infusion (BHI) with 5% Fungizone (amphotericin B), and 100μl of the sample was placed on a chocolate agar plate following the protocol published in Table 1. Prevalence of *Bartonella* infection across bat species, collection locations, and sexes. Confidence intervals were calculated using the Agresti-Coull method.

| Species                  | Family          | Captured | Tested | Positive | Positive (%) | 95% CI          | Coinfections |
|--------------------------|-----------------|----------|--------|----------|--------------|-----------------|---------------|
| *Eptesicus serotinus*    | Vespertilionidae| 20       | 20     | 4        | 20.0         | [7.5, 42.2]     | 0             |
| *Miniopterus schreibersii* | Miniopterae     | 29       | 27     | 24       | 88.9         | [71.1, 97]      | 7             |
| *Myotis blythii*         | Vespertilionidae| 75       | 67     | 32       | 47.8         | [36.2, 59.5]    | 3             |
| *Myotis emarginatus*     | Vespertilionidae| 42       | 38     | 15       | 39.5         | [25.6, 55.3]    | 1             |
| *Pipistrellus pygmaeus*  | Vespertilionidae| 13       | 12     | 2        | 16.7         | [3.5, 46]       | 0             |
| *Rhinolophus euryale*    | Rhinolophidae   | 29       | 26     | 18       | 69.2         | [49.9, 83.7]    | 2             |
| *Rhinolophus ferrumequinum* | Rhinolophidae  | 27       | 22     | 10       | 45.5         | [26.9, 65.4]    | 3             |

| Location                  | Habitat          | Captured | Tested | Positive | Positive (%) | 95% CI          | Species distribution |
|---------------------------|------------------|----------|--------|----------|--------------|-----------------|----------------------|
| Davit Gareja, Tetri Senakebi | 41.53603  45.257048 | 25       | 21     | 11       | 52.4         | [32.4, 71.7]     | 13 ME, 12 RF          |
| Davit Gareja, John the Baptist Cave | 41.298611 45.704722 | 25       | 24     | 15       | 62.5         | [42.6, 78.9]     | 25 MB                |
| Davit Gareja, Lavra       | 41.444742 45.376472 | 8        | 6      | 1        | 16.7         | [1.1, 58.2]      | 1 MB, 7 RF           |
| Gardabani Managed Reserve | 41.37699 45.0791 | 50       | 46     | 14       | 30.4         | [19, 44.9]       | 20 ES, 15 ME, 1 MM, 13 PP, 1 RF |
| Martvili, Leskulukhis Cave | 42.52927 42.10283 | 22       | 21     | 13       | 61.9         | [40.8, 79.3]     | 15 RE, 7 RF          |
| Terjola, Dzeveri, Bzebi Restaurant Cave | 42.183333 42.933333 | 20       | 18     | 10       | 55.6         | [33.7, 75.5]     | 5 MS, 15 MB          |
| Tskaltubo, Gliana Cave    | 42.37302 42.59749 | 53       | 48     | 31       | 64.6         | [50.4, 76.6]     | 18 MS, 26 MB, 9 RE   |
| Chkhorotsku, Letsurtsume Cave | 42.10375 42.32454 | 33       | 28     | 10       | 35.7         | [20.6, 54.2]     | 6 MS, 8 MB, 14 ME, 5 RE |
| Western Georgia, total    |                  | 106      | 94     | 51       | 54.3         | [44.2, 64]       | 29 MS, 49 MB, 14 ME, 14 RE |

| Sex           | Captured | Tested | Positive | Positive (%) | 95% CI       |
|---------------|----------|--------|----------|--------------|-------------|
| Female        | 177      | 160    | 73       | 45.6         | [38.1, 53.4] |
| Male          | 59       | 50     | 30       | 60.0         | [46.2, 72.4] |
| All total     | 236      | 212    | 105      | 49.5         | [42.9, 56.2] |

Two positive *Myotis blythii* were not sexed. Species abbreviations: ES–*Eptesicus serotinus*, MB–*Myotis blythii*, ME–*Myotis emarginatus*, MS–*Miniopterus schreibersii* sensu lato, PP–*Pipistrellus pygmaeus*, RE–*Rhinolophus euryale*, RF–*Rhinolophus ferrumequinum*.

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Tbilisi where they were stored at -80˚C until they were shipped on dry ice to the CDC’s laboratory, Fort Collins, Colorado. Upon arrival at CDC, the samples were stored at -80˚C until they were analyzed.
previously [27]. Inoculated plates were incubated at 35˚C in a 5% CO2 environment for up to five weeks. Plates were checked periodically, and bacterial colonies that morphologically resembled those typical for *Bartonella* were passaged onto a new plate to obtain pure cultures. In an attempt to capture possible *Bartonella* coinfections, all morphologically unique colonies growing from a single sample were sub-passaged and sequenced. All resulting isolates were collected in a 10% glycerol solution. Crude DNA extracts were obtained from isolates by heating a heavy suspension of the microorganisms for 10 minutes at 95˚C. Polymerase chain reactions (PCR) with the **gltA** primers BhCS781.p (5'-GGGGACCAGCTCATGGTG-3') and BhCS1137.n (5'-AATGCAAAAAGAACAATGAACCT-3') [28] were performed using PCR Thermal Cycler Dice (Takara Bio Inc., Japan) and C1000 Touch Thermal Cycler (Bio-Rad, Berkeley, CA). Positive (*B. doshiae*) and negative (nuclease free water) control samples were included in each PCR assay to evaluate the presence of appropriately sized amplicons and to rule out contamination of reagents, respectively. Positive PCR products were purified using QIAquick PCR purification Kit (Qiagen, Valencia, CA) and sequenced with an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Forward and reverse reads were assembled into consensus sequences with the SeqMan Pro program in Lasergene v. 11 (DNASTAR, Madison, WI).

**Phylogenetic analysis**

A BLAST ([http://blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)) search of the GenBank database was performed with all assembled **gltA** sequences to verify their *Bartonella* origin. Positive sequences were aligned with *Bartonella* reference sequences available in GenBank which included sequences obtained from various bats in previous studies. *Brucella abortus* sequence was used as outgroup. Alignment was performed with MAFFT v7.187 using the local, accurate L-INS-i method [29]. The optimal evolutionary model for the aligned sequences was determined by jModelTest2v2.1.6 [30] using Akaike information criterion corrected for finite sample sizes (AICc) for model selection [31]. For our dataset, the best model was the generalized time-reversible substitution model with four gamma-distributed categories and a proportion of invariant sites (GTR+Γ+I). We implemented this model for the Bayesian phylogeny of our sequences with BEAST v1.8.3 [32,33]. Since our goal was only to reconstruct the evolutionary topology of the sequences and not any demographic parameters, we assumed a constant population size for all branches. Similarly, we chose a strict molecular clock because the *Bartonella* sequences from Georgian bats were all isolated at the same date and thus could not be used for calibration of another clock model; furthermore, our analysis did not seek to accurately deduce branch times, and the strict clock was adequate. No codon partitioning was used due to the fact that **gltA** sequences represent only a 367 base pair fragment of the entire gene; codon partitioning with limited genetic information can substantially reduce the effective sample size of estimated parameters for separate codon positions [34]. All priors were kept at the default, diffuse settings (see Appendix) and the number of Markov chain Monte Carlo (MCMC) iterations was set to 1.2E8 with states sampled every 1.2E4 steps. Three independent chains were run and effective sample sizes and convergence of parameters during MCMC sampling were assessed using Tracer v1.6 [32]. TreeAnnotator was used to find the most probable tree with burning 10% of the initial trees. The selected tree was then visualized and edited in FigTree v1.4.2 [35]. Sequence alignment with MAFFT and phylogenetic analysis with BEAST were run using XSEDE supercomputing resources [36], accessed through the CIPRES Science Gateway [37]. A quantitative threshold for demarcation of sequences into genogroups was set at 96% nucleotide identity following recommendations by La Scola et al. proposed for demarcation of *Bartonella* species [38]. Based on this clustering scheme, branches on the phylogenetic tree
were collapsed and annotated with the number of sequences included in each genogroup and the range of DNA identity values.

Multi-locus typing of reference strains

Five genetic loci (ftsZ, gltA, nuoG, rpoB, and groEL) that have been previously used for bartonellacharacterization [9,39,40] were additionally investigated in 21 isolates representing 19 diverse genogroups identified based on variation of the gltA gene. Genogroups Vesp-7, Vesp-13, and Rhin-3 were not analyzed by MLST, while three isolates of Vesp-6 were selected for analysis to examine within-genogroup variation. The primers and cycle conditions used to generate sequences for each loci have been previously published [28,41–44]. Sequences were aligned with those of the reference Bartonella species and other Bartonella sequences obtained from bats with MAFFT v7.187 using the L-INS-i method [29]. Evolutionary model selection was performed for each marker separately and for the concatenated sequences using jModelTest2 v2.1.6 [30] based on AICc [31]. Again, the best available model for all sequences was GTR+Γ+i. A Bayesian tree was inferred using BEAST v1.8.3 [33] with the same settings and resources as for the gltA tree as described above. Separate maximum likelihood gene trees were generated using the GTRCAT model in RAxML [45]. A network phylogeny was created using the NeighborNet algorithm in SplitsTree v4.13.1 [46] and the pairwise homoplasy index [47] was calculated to test for evidence of recombination among genogroups. All unique sequences were uploaded to GenBank with accession numbers KX300105-KX300201 (Table 2).

Accession numbers

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.f0k4j
Statistical analysis

A logistic model was used to examine important predictors of Bartonella prevalence in Georgian bats. For this analysis, we included such variables as bat species, sex, capture location, and capture region. Additional size measurements (weight and forearm length), were collapsed into a single principle component that explained 95% of variation in size. However, bat size was strongly predicted by bat species ($F = 534.6$, p-value = 2E-16) and sex ($F = 25$, p-value = 1.3E-6), so size was not included as a covariate in the global model. Model selection was based on AICc [31]. Additional tests, including Wald tests of fixed effects and calculation of the area under the receiver operating characteristic curve (AUC), were performed on models within two AICc of the top model ($\Delta$AICc) [48,49]. Binomial confidence intervals for Bartonella prevalence among bat species, capture locations, and bat sexes were approximated with the Agresti-Coull method [50]. All statistical tests were performed in R [51] and values were considered significant for $P < 0.05$. Additional details of the statistical tests can be found in the Appendix.

Results

Bat species by site

A total of 236 bats were sampled from eight field sites in four regions of Georgia. The sampled bats included eight species: common serotine, Eptesicus serotinus (Vespertilionidae; n = 20); Schreibers’s long-fingered bat, Miniopterus schreibersii sensu lato (Miniopteridae; n = 29) [52]; lesser mouse-eared myotis, Myotis blythii (Vespertilionidae; n = 75); Geoffroy’s myotis, Myotis emarginatus (Vespertilionidae; n = 42); whiskered myotis, Myotis mystacinus (Vespertilionidae; n = 1); soprano pipistrelle, Pipistrellus pygmaeus (Vespertilionidae; n = 13); Mediterranean horseshoe bat, Rhinolophus euryale (Rhinolophidae; n = 29); and greater horseshoe bat, Rhinolophus ferrumequinum (Rhinolophidae; n = 27). The number of species and specimens obtained varied per site (Table 1).

Prevalence of Bartonella infections in bats

A total of 212 bats of seven species were available for Bartonella testing. The amount of blood from the single captured Myotis mystacinus was not sufficient for culturing. Except for this, bartonellae were successfully cultured from all bat species tested (Table 1). Bartonella colonies became visible within 3–28 days after plating. All plates remained free of contamination for the entire five week period and only Bartonella-like colonies were observed. Most of the isolated colonies appeared small, circular, and raised, with smooth or rough morphology. The number of Bartonella-like colonies observed per plate ranged from 1 colony to “too numerous to count” (TNTC). All the harvested colonies were confirmed as Bartonella by PCR and sequencing of gltA fragments. The overall prevalence of Bartonella in bats by culturing was 49.5% (105/212). Bartonella isolates were obtained from each of the eight sites. The prevalence of culture-positive bats varied from 16.7% at the Lavra site in Davit Gareja to 64.6% at Gliana Cave in Tskaltubo.

The range of prevalence varied from 16.7% in P. pygmaeus to 88.9% in M. schreibersii. The best model based on AICc included bat species only with a good amount of predictive power (AUC = 0.71) [49]. Based on the Wald test, there were significant differences among bat species ($\chi^2 = 26.9$, df = 6, p-value = 1.5E4) in Bartonella prevalence. Prevalence of Bartonella in Myotis blythii (odds ratio = 3.4, 95% CI = [1.1, 13], p-value = 0.044), Miniopterus schreibersii (odds ratio = 30.7, 95% CI = [6.9, 188.4], p-value = 3.7E-5), and R. euryale (odds ratio = 9, 95% CI = [2.4, 40], p-value = 0.0017) was significantly higher.
Coinfections

Culture observations from 16 bat samples revealed morphology differences among bacterial colonies. From these samples, Bartonella-like colonies were observed with morphologies that visually varied by size (small, large) and/or texture (rough, smooth). The number of visually different colonies per plate varied from one unique colony among TNTC similar colonies to an equal number of two unique colony morphologies. We did not attempt to estimate colony forming units (CFU) for individual bats suspected of coinfection. Sequencing analysis confirmed a coinfection with two different Bartonella sequences from these 16 samples (Table 1). Of those, seven were detected in Mn. schreibersii, three in My. blythii, one in My. emarginatus, two in R. euryale, and three in R. ferrumequinum (Table 1).

Phylogeny based on gltA sequences

The Bayesian analysis indicated that most gltA sequences from Georgian bats cluster closely with eachother as distinct genogroups from known Bartonella species. Based on a sequence identity threshold of 96%, we identified 22 distinct genogroups. Nucleotide sequence identity values varied between 97–100% within the identified genogroups. (Fig 1)

Results from BLAST searches for each Bartonella genogroup from Georgian bats are compiled in Table 3.

In some cases, Georgian bat sequences matched very closely with other bartonella sequences from related bats (same genus or family), but from distant locations. Other sequences, notably from genogroups Mini-1.1, Mini-3, and Vesp-6, clustered with bartonella sequences identified in dogs from Thailand [53] and in humans (forest workers) from Poland [54].

Phylogeny based on multiple loci

The phylogeny based on concatenated sequences from five genetic loci (ftsZ, gltA, nuoG, rpoB, and groEL) confirmed that most Bartonella genogroups from Georgian bats formed well-supported clades (posterior probability > 90%) with other Bartonella genogroups identified in bats. (Fig 2)

Genogroups Mini-1, Mini-1.1, Mini-2, Mini-3, Rhin-2, Rhin-4, Rhin-5, and Vesp-10 formed a well-supported clade with other Bartonella genogroups found in African pteropodid (Eidolon helvum and Rousettus aegyptiacus) [7,9], hipposiderid (Hipposideros sp. and Triniaops persicus) [7], and emballonurid (Coleura africana) [7] bats. Genogroups Mini-1 and Mini-1.1 clustered with another Bartonella genogroup found in Miniopterus schreibersii from Taiwan [13]. Genogroups Vesp-6, Vesp-8, Vesp-9, and Vesp-11 formed a second clade related to Candidatus Bartonella naantaliensis found in Myotis daubentonii from Finland [5]. These two clades were linked together by a node in the phylogeny; however, the posterior probability support for this node was only 53%.

Genogroups Rhin-1, Vesp-4, and Vesp-5 clustered with genogroup Ew from Eidolon helvum [7]. Genogroups Vesp-1, Vesp-2, and Vesp-3 clustered with Bartonella mayotimonensis isolated from a human endocarditis patient [26] and from European vespertilionid bats (Eptesicus nilssonii and Myotis daubentonii) [5]. These two clades were linked by a node, including Bartonella vinsonii subspecies, with low posterior probability support (50%). Finally, genogroup Vesp-12 clustered with genogroup E4 from Eidolon helvum [9], as well as with Bartonella claridgeiae and Bartonella rochalimae. The network phylogeny (Fig 3) indicated that most genogroups form distinct lineages, although there is some reticulation among related genogroups. In these cases, homologous recombination might be occurring among genogroups infecting a single bat species or a group of species. However, the pairwise homoplasy
index [47] did not indicate significant evidence for recombination (mean = 0.6, variance = 1.7E-5, p-value = 0.5), suggesting that the reticulations in the network did not have a strong influence on the evolutionary history of these genogroups.

Discussion

This report is the first to describe the prevalence, geographic patterns, and genetic characteristics of *Bartonella* species found in bat communities within the southern Caucasus. Several
Table 3. BLAST search results for *gltA* sequences from each *Bartonella* genogroup from Georgian bats.

| Genogroup | Total | Per host (n) | GenBank accession number | Source | Location | Sequence nucleotide identity (%) |
|-----------|-------|-------------|--------------------------|--------|----------|----------------------------------|
| Mini-1    | 14    |             | HM545139, KT751153       | Miniopterus sp., *Penicillidia leptothrinax* collected from *Miniopterus griveaudi* | Kenya, Madagascar | 100, 96 |
|           |       | *Mn. schreibersii* (11) |             |        |          |                                  |
|           |       | *E. serotinus* (1) |             |        |          |                                  |
|           |       | *My. blythii* (1) |             |        |          |                                  |
|           |       | *P. pygmaeus* (1) |             |        |          |                                  |
| Mini-1.1  | 8     | *Mn. schreibersii* (7) | FJ946852, JF500511 | Dog, *Miniopterus schreibersii* | Thailand, Taiwan | 99, 98 |
|           |       | *R. euryale* (1) |             |        |          |                                  |
| Mini-2    | 7     | *Mn. schreibersii* (7) | KT751143 | *Penicillidia leptothrinax* collected from *Miniopterus aelleni* | Madagascar | 98 |
| Mini-3    | 6     | *Mn. schreibersii* (6) | KT751152, FJ946854, HM545140 | *Nycteribia styliodipsis* collected from *Miniopterus gleni*, dog, *Miniopterus sp.* | Madagascar, Thailand, Kenya | 100, 99, 99 |
| Rhin-1    | 4     | *R. ferrumequinum* (3) | AF470616 | *Spermophilus beecheyi* | US | 95 |
|           |       | *My. emarginatus* (1) |             |        |          |                                  |
| Rhin-2    | 2     | *R. ferrumequinum* (2) | KP100344, KP100345 | *Rhinolophus sinicus, Rhinolophus acuminatus* | Vietnam | 98, 97 |
| Rhin-3    | 5     | *My. blythii* (2) | KP100342, KP100344 | *Rhinolophus sinicus, Rhinolophus acuminatus* | Vietnam | 96, 95 |
|           |       | *R. euryale* (1) |             |        |          |                                  |
|           |       | *R. ferrumequinum* (2) |             |        |          |                                  |
| Rhin-4    | 17    | *R. euryale* (13) | JX416255, JX416239, KP100350 | *Cyclopodia simulans* collected from *Phonochirus jagori, Leptocyclopodia sp.* collected from *Harpionycteris whiteheadi, Rhinolophus acuminatus* | Philippines, Philippines, Vietnam | 92, 92, 91 |
|           |       | *R. ferrumequinum* (3) |             |        |          |                                  |
|           |       | *Mn. schreibersii* (1) |             |        |          |                                  |
| Rhin-5    | 9     | *R. euryale* (6) | KP100355 | *Hipposideros larvatus* | Vietnam | 95 |
|           |       | *R. ferrumequinum* (3) |             |        |          |                                  |
| Vesp-1    | 2     | *My. emarginatus* (2) | KF003137, AJ871614 | Bat flea collected from vesperilionid bat, *Pipistrellus sp.* | Finland, UK | 99, 98 |
| Vesp-2    | 1     | *My. blythii* (1) | KF003122 | *Myotis daubentonii* | UK | 99 |
| Vesp-3    | 1     | *E. serotinus* (1) | KF003115, AJ871612 | *Eptesicus nilssonii, Myotis mystacinus* | Finland, UK | 99, 98 |
| Vesp-4    | 5     | *My. blythii* (5) | KJ816667 | *Anatrichoius scorzar* collected from *Myotis keaysi* | Costa Rica | 94 |
| Vesp-5    | 1     | *My. blythii* (1) | KJ816667 | *Anatrichoius scorzar* collected from *Myotis keaysi* | Costa Rica | 94 |
| Vesp-6    | 18    | *My. blythii* (15) | JQ695834, KR822802, HM116785 | *Myotis myotis, Myotis daubentonii, human* | Poland, Finland, Poland | 100, 99, 99 |
|           |       | *E. serotinus* (1) |             |        |          |                                  |
|           |       | *My. emarginatus* (2) |             |        |          |                                  |
| Vesp-7    | 4     | *E. serotinus* (1) | JQ695834, KR822802, HM116785 | *Myotis myotis, Myotis daubentonii, human* | Poland, Finland, Poland | 99, 97, 98 |
|           |       | *My. emarginatus* (3) |             |        |          |                                  |
| Vesp-8    | 4     | *My. blythii* (3) | JQ695834, KR822802, HM116785 | *Myotis myotis, Myotis daubentonii, human* | Poland, Finland, Poland | 96, 96, 96 |
|           |       | *My. emarginatus* (1) |             |        |          |                                  |
|           |       | *Mn. schreibersii* (1) |             |        |          |                                  |
| Vesp-9    | 8     | *My. emarginatus* (6) | KF003129, KJ816689 | *Myotis daubentonii, Basilia sp.* collected from *Myotis keaysi* | Finland, Costa Rica | 93, 91 |
|           |       | *My. blythii* (1) |             |        |          |                                  |
| Vesp-10   | 3     | *My. blythii* (3) | JX416246, JX416241, KT751152 | *Basilia coronata* collected from *Tyto alba*, *Basilia nattereri* collected from *Myotis nattereri, Nycteribia styliodipsis* collected from *Miniopterus gleni* | Thailand, Malaysia, Slovenia, Madagascar | 98, 98, 97 |

(Continued)
interesting conclusions can be drawn from the study. First, we provided the evidence that Bartonella infections are widespread and highly prevalent in all seven bat species tested. This observation is comparable to the investigations of Bartonella species in bats from other geographic regions (e.g., Kenya, Guatemala, and Peru) where high prevalence and diversity of Bartonella strains have been reported [7,15,16]. However, in our study the prevalence of infection varied greatly between bat species (nearly 89% in Mn. schreibersii and below 17% in P. pygmaeus) as well as between study sites. The difference in prevalence between locations can be likely explained by bat community composition (Table 1). For example, P. pygmaeus was only captured at one location whereas Mn. schreibersii was collected from many sites, and the bat colony at John the Baptist Cave in Davit Gareja consisted solely of My. blythii. (Fig 4).

These sampling biases should be considered when interpreting Bartonella prevalence values. We also cannot exclude other factors, including the level of ectoparasite infestation in bats that may influence the prevalence of Bartonella in each bat species and locations.

We observed several coinfections among sampled bats. The phenomenon of coinfections with two or three different Bartonella species or genotypes in blood has been described previously for rodents [55]. Interestingly, a high rate of coinfection was observed in one particular bat species, Mn. schreibersii. Seven of the 27 (26%) Mn. schreibersii tested were coinfected with two different Bartonella genotypes (Patterns of codivergence of Bartonella with their bat hosts have varied among studies and around the world [7,15,16,20]. For Bartonella genogroups found in Georgian bats, some general patterns of host specificity at the genus and family level are apparent. Nearly all of the isolates (33/35) from Mn. schreibersii aligned with genogroups Mini-1, Mini-1.1, Mini-2, or Mini-3 (Table 3). Based on sequence identity at the gltA gene, all of these genogroups closely matched Bartonella sequences from other Miniopterus spp. (e.g., Mn. griveaudi, Mn. aelleni, and Mn. gleni) from Madagascar [11]. Thirty-seven of 38 isolates obtained from Rhinolophus spp. (R. euryale or R. ferrumequinum) belonged to genogroups Rhin-1, Rhin-2, Rhin-3, or Rhin-4. Genogroups Rhin-2 and Rhin-3 cluster with Bartonella sequences identified in R. acuminatus and R. sinicus from Vietnam [14]. Most isolates (54/60) obtained from vespertilionid bats (Epitesicus, Myotis, and Pipistrellus spp.) were members of genogroups Vesp-1 to Vesp-12 with closely matching sequences found in other vespertilionid bats [4–6,17,56].

Despite these general host associations, specificity of genogroups at the genus or family level was not strict, with some instances of apparent spillover of Bartonella into atypical hosts. For example, isolates of Bartonella from genogroup Mini-1 were found in E. serotinus, My. blythii, and P. pygmaeus, and isolates of Bartonella from genogroups Rhin-1 and Rhin-3 were found in My. emarginatus and My. blythii, respectively (Table 3). Though infrequent, these spillover events can be explained by the co-occurrence of these bat species in the same roosts.

Table 3. (Continued)

| Georgian bats | GenBank accession number | Source | Location | Sequence nucleotide identity (%) |
|---------------|--------------------------|--------|----------|----------------------------------|
| Vesp-11       | KT751154                 | Penicillidia cf. fulvida collected from Miniopterus griveaudi | Madagascar | 92 |
| Vesp-12       | KM030517, GU056189       | Eidolon helvum, human | Africa, Thailand | 91, 92 |
| Vesp-13       | KT751145, JX416252       | Penicillidia leptothrinax collected from Miniopterus manavi, Phthiridium sp. scissa group collected from Rhinolophus pearsoni | Madagascar, Laos | 97, 95 |

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transmission may be facilitated by shared vectors. Ectoparasites were collected from bats at the sampled sites in Georgia in 2012, but have not yet been identified and are thus not included in this study. However, there are numerous ectoparasite species reported on our seven focal bat species in the literature. While some ectoparasite species preferentially feed on specific bat hosts, they can also be found infrequently on other bat hosts, which may lead to transmission of bacteria. For example, bat flies (Diptera: Nycteribiidae) can be closely associated with one or a few bat hosts: Basilia nana with Myotis bechsteinii [57], Basilia nattereri with Myotis nattereri [58], Nycteribia schmidlii and Penicillidia conspicua with Miniopterus schreibersii [59], and Phthiridium biarticulatum with Rhinolophus spp. [60]. Nevertheless, there are recorded incidents of these bat flies on other bat hosts, including the focal species in this study: Basilia nana recorded on My. blythii and My. emarginatus [61], Basilia nattereri...
recorded on *E. serotinus* [62], *Nycteribia schmidii* recorded on *My. blythii, My. emarginatus, R. euryale, and R. ferrumequinum* [61,63], *Penicillidia conspicua* on *My. blythii* [61], and *Phthiridium biarticulatum* on *E. serotinus, Mn. schreibersii, and My. emarginatus* [61,64]. Other ectoparasites can have broader and more evenly distributed host ranges, and may be found infesting our focal bat species. *Argas vespertilionis* (Ixodida: Argasidae) has been collected from *E. serotinus, My. blythii, P. pygmaeus, and R. ferrumequinum* [61,65,66]. *Cimex pipistrelli* (Hemiptera: Cimicidae) has been reported parasitizing *E. serotinus, My. blythii, My. emarginatus, P. pygmaeus, and R. ferrumequinum* [67,68]. Additionally, *Spinturnix myoti* (Mesostigmata: Spinturnicidae) has been recorded on *E. serotinus, Mn. schreibersii, My. blythii, R. euryale, and R. ferrumequinum* [69–71]. This short review of the literature is not exhaustive, but is meant to illustrate that nonspecific parasitism by *Bartonella* genogroups in some bat hosts can potentially be explained by sharing of ectoparasites. Future analyses exploring the influence of ectoparasite distributions on sharing of *Bartonella* genogroups among bats are in progress.

The sequence characterization of five house-keeping genes (*ftsZ, gltA, nuoG, rpoB*, and *groEL*) along with the network phylogenetic analysis strongly indicated that many genogroups characterized in our study can be segregated into new *Bartonella* species according to
established demarcation criteria considering loci separately [38], with sequence identity >95% based on concatenated loci for most pairwise comparisons within each *Bartonella* genogroup. The host associations observed for most of identified genetic clusters also supports the biological basis for discrimination of the species. As was reasoned previously [72], a refined approach that combines data from multiple genetic markers with ecological information about host specificity provides more reliable and tangible demarcations of *Bartonella* species compared to sequence analysis alone. For example, genogroups Vesp-1, Vesp-2, and Vesp-3 share 92%, 93%, and 92% nucleotide identity, respectively, with *Bartonella mayotimonensis*, the bacterial species discovered in a human patient in the United States [26]. However, *B. mayotimonensis* is closest (95%) at the *gltA* locus to a sequence identified in a bat fly *Anatrichobius scorzai* taken from a bat *Myotis keaysi* in Costa Rica [17]. It is likely that clusters Vesp-1, Vesp-2, Vesp-3, and the bat fly strain from Costa Rica can be assigned to the *B. mayotimonensis* species, but using the *gltA* locus alone creates an artifactual split among the genogroups. When all five concatenated loci were considered, genogroups Vesp-1, Vesp-2, and Vesp-3 shared pairwise sequence identities between 96.9–98.11%. Considering their relatedness and apparent specificity to vespertilionid bats (*Eptesicus*, *Myotis*, and *Pipistrellus* spp.) [5], all of these genogroups may be included as one species. The pairwise identities of these genogroups with *B. mayotimonensis* ranged 95.1–95.5%, which is near the previously established minimum threshold for distinguishing between *Bartonella* species (95.4% for *rpoB* sequences [38]) and we argue it should be considered synonymous with Vesp-1, Vesp-2, and Vesp-3. Similarly, genogroups Vesp-6 and Vesp-8 were 95.9% identical and considering their apparent specificity to vespertilionid bats (*Eptesicus* and *Myotis*) [5] they may also constitute a single *Bartonella* species. This is also true for genogroups Vesp-4 and Vesp-5 found in one bat species, *My. blythii* (96.3% sequence identity) and genogroups Mini-1 and Mini-1.1 found in *Mn. schreibersii* (96.6% sequence identity).
The most intriguing and important results from this study is the identification of bat-borne *Bartonella*, which are similar to *Bartonella* strains previously reported in humans and in dogs. The public health relevance of bat-borne *Bartonella* infection has been discussed since the identification of such bacteria in bats from Kenya [7]. Our results highlight the importance of *Bartonella* surveillance in bats, as it can help to identify potential wildlife reservoirs of human cases. Although some sequences of *Bartonella* found in Georgian bats clustered with *B. mayotimonensis*, the genetic distances were relatively long, as noted above. We might speculate that *Bartonella* more closely related to this human case are circulating in vespertilionid bats in the North and South America rather than in Europe. Even more unexpected was the discovery of *Bartonella* strains in Georgian bats which were identical or very similar to ones reported in forest workers from Poland. The study in Poland was conducted to evaluate the level of exposure of 129 forest workers to diverse tick-borne pathogens [54]. *Bartonella* antibodies were reported in about 30% of tested individuals, but more importantly, three serologically-positive samples were also positive for *Bartonella* nucleic acids by PCR and sequencing. The *gltA* sequences identified in that study were distinct from all previously reported. They were closest (90% similarity) to *B. koehlerae*, *B. clarridgeiae* and a genotype from an arthropod from Peru. They were deposited in GenBank (accessions HM116784, HM116785, and HM116786) as uncultured *Bartonella* spp. [54]. All strains identified in our study as genotype Vesp-6 were 100% identical by *gltA* sequences to the HM116785 sequence. Vesp-6 is the largest genogroup found in bats from Georgia containing 18 sequences from *My. blythii* (*n* = 15), *My. emarginatus* (*n* = 2), and *E. serotinus* (*n* = 1). All of these bat species are listed as occurring in southern Poland where the investigation of forest workers was conducted [73–75].

Another surprising discovery was that *Bartonella* strains observed in this study were closely related to those identified in stray dogs from Thailand., Bai et al. [53] provided evidence of common *Bartonella* infections and diverse *Bartonella* species in the blood of stray dogs from Bangkok and Khon Kaen (northeastern province of Thailand). Besides two *Bartonella* species (*B. elizabethae* and *B. taylorii*) detected in stray dogs from Khon Kaen, the authors also reported two genotypes (KK20 and KK61) that could potentially represent a new species [53]. Performing the analysis of *Bartonella* strains found in bats from Georgia, we found that sequences of the strains from genogroup Mini-1.1 obtained from *Mn. schreibersii* (*n* = 7) and *R. euryale* (*n* = 1) were 99% similar to those dog sequences from Thailand (strain KK61, GenBank accession FJ946852). Likewise, seven sequences from *Mn. schreibersii* (genogroup Mini-3) were 99% similar to the sequences of the strain KK20 from stray dogs from Khon Kaen, Thailand (GenBank accession FJ946854). Bat species belonging to the genus Miniopterus (e.g., *Mn. magnater* and *Mn. pusillus*) are present in Thailand [76].

The identification of diverse *Bartonella* strains in Georgian bats, which are identical or similar to the strains previously described in humans and in companion animals in other geographic areas grants special attention in future studies to evaluate their role as potential zoonotic agents. A particular question remains regarding the route of transmission of bat-associated *Bartonella* to people. Its easier to speculate how stray dogs, which may scavenge for grounded bats, can become infected with bat-associated *Bartonella*, but the question concerning transmission of bat-borne strains to humans is more challenging [77]. However, the human case of endocarditis linked to a bat-associated *Bartonella* species [5,26] suggests that such transmission can occur. Some bat ectoparasites are known to occasionally bite humans, including *Argas vespertilionis* and *Cimex pipistrelli* [78–80]. Thus, *Bartonella* surveillance should include not only mammals, but also their vectors whenever possible to better understand the risks of disease transmission.
Supporting information
S1 Appendix.

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Author Contributions
Conceived and designed the experiments: LU YB PI MK.

Performed the experiments: LU YB LO CM AK IK KS DP MK.

Analyzed the data: LU YB LO CM AK IK PI MK.

Contributed reagents/materials/analysis tools: LU IK KS PI MK.

Wrote the paper: LU LO CM AK IK MK.

References
1. Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T (2006) Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 19: 531–545. https://doi.org/10.1128/CMR.00017-06 PMID: 16847084

2. Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, et al. (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proc R Soc London Ser B, Biol Sci 280: e20122753.

3. Mühldorfer K (2013) Bats and bacterial pathogens: a review. Zoonoses Public Health 60: 93–103. https://doi.org/10.1111/j.1663-2378.2012.01536.x PMID: 22862791

4. Concannon R, Wynn-Owen K, Simpson V, Birtles RJ (2005) Molecular characterization of haemoparasites infecting bats (Microchiroptera) in Cornwall, UK. Parasitology 131: 489–496. https://doi.org/10.1017/S0031182005008097 PMID: 16174413

5. Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT (2014) Bats as reservoir hosts of human bacterial pathogen, Bartonella mayotimonensis. Emerg Infect Dis 20: 960–967. https://doi.org/10.3201/eid2006.130956 PMID: 24856523

6. Lilley TM, Veikkolainen V, Pulliainen AT (2015) Molecular detection of Candidatus Bartonella hemsundtiensis in bats. Vector-Borne Zoonotic Dis 15: 706–708. https://doi.org/10.1089/vbz.2015.1783 PMID: 26501463

7. Kosoy MY, Bai Y, Lynch T, Kuzmin I V, Niezgoda M, et al. (2010) Bartonella spp. in bats, Kenya. Emerg Infect Dis 16: 1875–1881. https://doi.org/10.3201/eid1612.100601 PMID: 21122216

8. Kamani J, Baneth G, Mitchell M, Mumcuoglu KY, Gutiérrez R, et al. (2014) Bartonella species in bats (Chiroptera) and bat flies (Nycteribiidae) from Nigeria, West Africa. Vector-Borne Zoonotic Dis 14: 625–632. https://doi.org/10.1089/vbz.2013.1941 PMID: 25229701

9. Bai Y, Hayman DT, McKee CD, Kosoy MY (2015) Classification of Bartonella strains associated with straw-colored fruit bats (Eidolon helvum) across Africa using a multi-locus sequence typing platform. PLoS Negl Trop Dis 9: e0003478. https://doi.org/10.1371/journal.pntd.0003478 PMID: 25635826

10. Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranalvoson HC, et al. (2015) Bartonella spp. in fruit bats and blood-feeding ectoparasites in Madagascar. PLoS Negl Trop Dis 9: e0003532. https://doi.org/10.1371/journal.pntd.0003532 PMID: 25706653

11. Wilkinson DA, Duron O, Cordonin C, Gornard Y, Ramasindrazana B, et al. (2016) The bacteriome of bat flies (Nycteribiidae) from the Malagasy region: a community shaped by host ecology, bacterial
33. Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29: 1969–1973. https://doi.org/10.1093/molbev/ms2075 PMID: 22367748
34. Hayman DT, McDonald KD, Kosoy MY (2013) Evolutionary history of rat-borne *Bartonella*: the importance of commensal rats in the dissemination of bacterial infections globally. Ecol Evol 3: 3195–3203. https://doi.org/10.1002/ece3.702 PMID: 24223261
35. Rambaut A (2014) FigTree v1.4.2. http://tree.bio.ed.ac.uk/software/figtree/
36. Towns J, Cockerill T, Dahan M, Foster I, Gaither K, et al. (2014) XSEDE: accelerating scientific discovery. Comput Sci Eng 16: 62–74.
37. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE). pp. 1–8.
38. La Scola B, Zeaiter Z, Khamis A, Raoult D (2003) Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. Trends Microbiol 11: 318–321. PMID: 12875815
39. Bai Y, Malania L, Alvarez Castillo D, Moran D, Boonmar S, et al. (2013) Global distribution of *Bartonella* infections in domestic bovine and characterization of *Bartonella bovis* strains using multi-locus sequence typing. PLoS One 8: e80894. https://doi.org/10.1371/journal.pone.0080894 PMID: 24278342
40. Buffet J-P, Pisanu B, Brisse S, Rousseau S, Félix B, et al. (2013) Deciphering *Bartonella* diversity, recombination, and host specificity in a rodent community. PLoS One 8: 13671. https://doi.org/10.1371/journal.pone.0068956 PMID: 23894381
41. Zeaiter Z, Liang Z, Raoult D (2002) Phylogenetic classification of *Bartonella* species by comparing *groEL* sequences. Int J Syst Evol Microbiol 52: 165–171. https://doi.org/10.1099/00207713-52-1-165 PMID: 11837299
42. Renesto P, Gouvernet J (2001) Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. J Clin Microbiol 39: 430–437. https://doi.org/10.1128/JCM.39.2.430-437.2001 PMID: 11158086
43. Zeaiter Z, Fournier P-E, Ogata H, Raoult D (2002) Phylogenetic classification of *Bartonella* species by comparing *rpoB* gene sequences. J Clin Microbiol 40: 3641–3647. https://doi.org/10.1128/JCM.40.10.3641-3647.2002 PMID: 12354859
44. Colborn JM, Kosoy MY, Motin VL, Telepnev M V, Valbuena G, et al. (2010) Improved detection of *Bartonella* DNA in mammalian hosts and arthropod vectors by real-time PCR using the NADH dehydrogenase gamma subunit (*nuoG*). J Clin Microbiol 48: 4630–4633. https://doi.org/10.1128/JCM.00470-10 PMID: 20926707
45. Buffet J-P, Pisanu B, Brisse S, Rousseau S, Félix B, et al. (2013) Deciphering *Bartonella* diversity, recombination, and host specificity in a rodent community. PLoS One 8: 13671. https://doi.org/10.1371/journal.pone.0068956 PMID: 23894381
46. Zeaiter Z, Fournier P-E, Ogata H, Raoult D (2002) Phylogenetic classification of *Bartonella* species by comparing *groEL* sequences. Int J Syst Evol Microbiol 52: 165–171. https://doi.org/10.1099/00207713-52-1-165 PMID: 11837299
47. Renesto P, Gouvernet J (2001) Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. J Clin Microbiol 39: 430–437. https://doi.org/10.1128/JCM.39.2.430-437.2001 PMID: 11158086
48. Colborn JM, Kosoy MY, Motin VL, Telepnev M V, Valbuena G, et al. (2010) Improved detection of *Bartonella* DNA in mammalian hosts and arthropod vectors by real-time PCR using the NADH dehydrogenase gamma subunit (*nuoG*). J Clin Microbiol 48: 4630–4633. https://doi.org/10.1128/JCM.00470-10 PMID: 20926707
49. Buffet J-P, Pisanu B, Brisse S, Rousseau S, Félix B, et al. (2013) Deciphering *Bartonella* diversity, recombination, and host specificity in a rodent community. PLoS One 8: 13671. https://doi.org/10.1371/journal.pone.0068956 PMID: 23894381
50. Zeaiter Z, Fournier P-E, Ogata H, Raoult D (2002) Phylogenetic classification of *Bartonella* species by comparing *groEL* sequences. Int J Syst Evol Microbiol 52: 165–171. https://doi.org/10.1099/00207713-52-1-165 PMID: 11837299
51. Renesto P, Gouvernet J (2001) Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. J Clin Microbiol 39: 430–437. https://doi.org/10.1128/JCM.39.2.430-437.2001 PMID: 11158086
52. Bruen TC, Philippe H, Bryant D (2005) A simple and robust statistical test for detecting the presence of recombination. Genetics 172: 2665–2681.
53. Burnham K, Anderson D (2002) Model selection and multimodel inference: a practical information-theoretic approach, second edition. New York: Springer. 488 p.
54. Hosmer DW, Lemeshow S (2000) Applied logistic regression, second edition. New York: Wiley and Sons, Inc.
55. Agresti A (1990) Categorical Data Analysis. New York: Wiley and Sons, Inc.
56. R Core Team (2016) R: a language and environment for statistical computing. R Found Statistical Comput. Vienna, Austria. http://www.r-project.org.
57. Koopman K (1994) Chiroptera: systematics. New York: W. de Gruyter. 217 p.
58. Bai Y, Kosoy MY, Boonmar S, Sawatwong P, Sangmanevedet S, et al. (2010) Enrichment culture and molecular identification of diverse *Bartonella* species in stray dogs. Vet Microbiol 146: 314–319. https://doi.org/10.1016/j.vetmic.2010.05.017 PMID: 20570065
59. Podsiadly E, Chmielewski T, Karbowiak G, Kedra E, Tylewska-Wierzbanowska S (2010) The occurrence of spotted fever rickettsioses and other tick-borne infections in forest workers in Poland. Vector-Borne Zoonotic Dis 11: 985–989. https://doi.org/10.1089/vbz.2010.0080 PMID: 21083370
60. Kosoy MY, Mandel E, Green D, Marston E, Jones DC, et al. (2004) Prospective studies of *Bartonella* of rodents. Part II. Diverse infections in a single rodent community. Vector-Borne Zoonotic Dis 4: 296–305. https://doi.org/10.1089/vbz.2004.4.296 PMID: 15671736
56. Morse SF, Olival KJ, Kosoy MY, Billeter SA, Patterson BD, et al. (2012) Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). Infect Genet Evol 12: 1717–1723. https://doi.org/10.1016/j.meegid.2012.06.009 PMID: 22771358

57. van Schaik J, Dekeukeleire D, Kerth G (2015) Host and parasite life history interplay to yield divergent population genetic structures in two ectoparasites living on the same bat species. Mol Ecol 24: 2324–2335. https://doi.org/10.1111/mec.13171 PMID: 25809613

58. Kríštofi J, Danko S (2012) Arthropod ectoparasites (Acarina, Heteroptera, Diptera, Siphonaptera) of bats in Slovakia. Vespertilio: 167–189.

59. Lourenço S, Palmeirim JM (2008) Which factors regulate the reproduction of ectoparasites of temperate-zone cave-dwelling bats? Parasitol Res 104: 127–134. https://doi.org/10.1007/s00436-008-1170-6 PMID: 18779978

60. Scheffler I, Bego F, Théou P, Podany M, Pospischil R, et al. (2013) Ectoparasiten der Fledermäuse in Albanien—Artenspektrum und Wirtsbindung. Nyctalus 18: 84–109.

61. Frank R, Kuhn T, Werblow A, Liston A, Kochmann J, et al. (2015) Parasite diversity of European *Myotis* species with special emphasis on *Myotis myotis* (Microchiroptera, Vespertilionidae) from a typical nursery roost. Parasit Vectors 8: 101. https://doi.org/10.1186/s13071-015-0707-7 PMID: 25880235

62. Masson D (1989) Sur L'infestation de *Myotis nattereri* (Kuhl, 1818) (Chiroptera, Vespertilionidae) par *Basilia nattereri* (Kolenati, 1857) (Diptera, Nycteribiidae) dans le sud-ouest de la France. Ann Parasitol Hum Comp 64: 64–71.

63. Vanin S, Vernier E (2009) Contribution to the knowledge of the Nycteribiidae (Diptera) from Venetian Region. Parasitologia 51: 61–64.

64. Bendjeddou ML, Bitam I, Abiad A, Bouslama Z, Amr ZS (2013) New Records of Arthropod Ectoparasites of Bats from North—Eastern Algeria. Jordan J Biol Sci 6: 324–327.

65. Siuda K, Stanko M, Pińska K, Görz A (2009) Ticks (Acari: Ixodida) parasitizing bats in Poland and. Wiad Parazytol 55: 39–45. PMID: 19579784

66. Bursali A, Keskin A, Tekin S (2012) A review of the ticks (Acari: Ixodida) of Turkey: Species diversity, hosts and geographical distribution. Exp Appl Acarol 57: 91–104. https://doi.org/10.1007/s10493-012-9530-4 PMID: 22371208

67. Balvín O, Bartońčík T, Simov N, Paunovic M, Vilimova J (2014) Cimicids and bat hosts in the Czech and Slovak Republics: ecology and distribution. Vespertilio 17: 23–36.

68. Deunff J (1977) Observations sur les Spinturnicidae de la region Observations sur les Spinturnicidae de la Region Palearctique occidentale (Acarina: Mesostigmata): Specificite, repartition et morphologic. Acarologia 18: 602–617.

69. Deunff J, Walter G, Bellido A, Volleth M (2004) Description of a cryptic species, *Spinturnix bechsteini* n. sp. (Acar, Mesostigmatida, Spinturnicidae), parasite of *Myotis bechsteini* (Kuhl, 1817) (Chiroptera, Vespertilionidae) by using ecoethology of host bats and statistical methods. J Med Entomol 41: 826–832. PMID: 15535609

70. Ferenc H, Skoracki M (2000) Stan zbadani a roztoczy z rodziny spinturnicidae (Acari: Mesostigmata) w polsce. Wiad Parazytol 46: 433–438. PMID: 16886323

71. Kosoy MY, Hayman DT, Chan K-S (2012) *Bartonella* bacteria in nature: where does population variability end and a species start? Infect Genet Evol 12: 894–904. https://doi.org/10.1016/j.meegid.2012.03.005 PMID: 22449771

72. Wachara pluesadee S, Duengkae P, Rodpan A, Kaewpom T, Maneeorn P, et al. (2015) Diversity of coronavirus in bats from Eastern Thailand. Virol J 12: 57. https://doi.org/10.1186/s12985-015-0289-1 PMID: 25884446

73. Mannerings AO, Osikowicz LM, Restif O, Nyarko E, Suu-Ire R, et al. (2016) Exposure to bat-associated *Bartonella* spp. among humans and other animals, Ghana. Emerg Infect Dis 22: 922–924. https://doi.org/10.3201/eid2205.151908 PMID: 27088812
78. Jaenson TG, Tälleklint L, Lundqvist L, Olsen B, Chirico J, et al. (1994) Geographical distribution, host associations, and vector roles of ticks (Acaric: Ixodidae, Argasidae) in Sweden. J Med Entomol 31: 240–256. PMID: 8189415

79. Whyte A, Garnett P, Whittington A (2001) Bats in the belfry, bugs in the bed? Lancet 357: 604. PMID: 11558488

80. Socolovski C, Kernif T, Raoult D, Parola P (2012) Borrelia, Rickettsia, and Ehrlichia Species in Bat Ticks, France, 2010. Emerg Infect Dis 18: 1966–1975. https://doi.org/10.3201/eid1812.111237 PMID: 23171714