RESEARCH ARTICLE

Genetic diversity, infection prevalence, and possible transmission routes of *Bartonella* spp. in vampire bats

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Abstract

*Bartonella* spp. are globally distributed bacteria that cause endocarditis in humans and domestic animals. Recent work has suggested bats as zoonotic reservoirs of some human *Bartonella* infections; however, the ecological and spatiotemporal patterns of infection in bats remain largely unknown. Here we studied the genetic diversity, prevalence of infection across seasons and years, individual risk factors, and possible transmission routes of *Bartonella* in populations of common vampire bats (*Desmodus rotundus*) in Peru and Belize, for which high infection prevalence has previously been reported. Phylogenetic analysis of the *gltA* gene for a subset of PCR-positive blood samples revealed sequences that were related to *Bartonella* described from vampire bats from Mexico, other Neotropical bat species, and streblid bat flies. Sequences associated with vampire bats clustered significantly by country but commonly spanned Central and South America, implying limited spatial structure. Stable and nonzero *Bartonella* prevalence between years supported endemic transmission in all sites. The odds of *Bartonella* infection for individual bats was unrelated to the intensity of bat flies ectoparasitism, but nearly all infected bats were infested, which precluded conclusive assessment of support for vector-borne transmission. While metagenomic sequencing found no strong evidence of *Bartonella* DNA in pooled bat saliva and fecal samples, we detected PCR positivity in individual saliva and feces, suggesting the potential for bacterial transmission through both direct contact (i.e., biting) and environmental (i.e., fecal) exposures. Further investigating the relative contributions of direct contact, environmental, and vector-borne transmission for bat *Bartonella* is an important next step to predict infection dynamics within bats and the risks of human and livestock exposures.
Author summary

_Bartonella_ are globally distributed bacteria that can cause endocarditis in humans and domestic animals. Bats have been implicated as a likely reservoir host for these bacteria, but little is known about how prevalence varies over time, routes of transmission, and the genetic diversity of _Bartonella_ in bats. We present results from a two-year, spatially replicated study of common vampire bats, which have previously shown high infection prevalence of _Bartonella_ and could pose risks of cross-species transmission due to their diet of mammal blood. We found that vampire bat _Bartonella_ is genetically diverse, geographically widespread and endemic, and that individual-level infection risk is highest for large, male, non-reproductive bats. Phylogenetic analysis supported vector-borne transmission, and we found support for potential transmission through direct contact and fecal exposures through PCR. Confirming whether arthropod vectors are the main route of transmission for bat _Bartonella_ is needed for understanding infection dynamics in bats and for predicting risks of cross-species transmission to humans and livestock.

Introduction

Bats (Order: Chiroptera) serve as reservoir hosts for viruses of concern for human and animal health [1,2] including SARS coronavirus, rabies virus, filoviruses, and henipaviruses [3–6]. Bats can also harbor protozoa and bacteria of potential zoonotic relevance [7–9]. _Bartonella_ spp. are of particular interest, as these Gram-negative bacteria cause bacteremia and endocarditis in both humans and livestock [10,11] and exhibit high genetic diversity in bats across multiple continents and species [12–17]. Moreover, phylogenetic analyses show bats are reservoirs of zoonotic _Candidatus_ B. mayotimonensis [18–20], a causative agent of human endocarditis [21].

Given the zoonotic potential of bat-associated _Bartonella_, understanding transmission within bats is critical for understanding how _Bartonella_ persists in bat populations and for assessing spillover risks [22,23]. Ectoparasites are frequently invoked as a transmission route [12,19,24], in part because vector-borne transmission occurs in other taxa [25,26] and because _Bartonella_ has been identified in bat flies and ticks [27–29]. While some bat ticks can feed on humans [30], the high host specificity of bat flies [31,32] could limit opportunities for cross-species transmission through ectoparasites [31–33]. Transmission through close contact (e.g., biting) could occur given detection of _Bartonella_ in dog and cat saliva [34,35] as well as human infection following scratches from dogs and cats [36]. Phylogenetic patterns of weak _Bartonella_ host specificity in Neotropical bat communities could not only reflect transmission through close contacts between species in multi-species roosts, but could also stem from transmission through generalist vectors [15,24,37]. _Bartonella_ might also be transmitted through exposure to feces between bats and to humans that enter roosts or to domestic animals exposed to bat feces [18,38].

In addition to the potential risks of cross-species transmission from bats to livestock and humans, the infection dynamics of _Bartonella_ in bats are also uncertain. In rodents, _Bartonella_ prevalence varies through time [39,40], but such patterns have not been well studied in bats [41]. Individual heterogeneities in infection by age and sex could also inform exposure patterns. Finally, global analyses suggest geographic structure in bat _Bartonella_ genotypes, with notable differences in genotypes from Latin American and those from Africa, Europe, and Asia [42]. However, as such patterns appear driven by bat families restricted to different continents, analyses within narrower geographic and taxonomic ranges could inform the scale of...
Bartonella transmission and the role that dispersal plays in the spatial dynamics of this infection [43].

Common vampire bats (Desmodus rotundus) have high prevalence of Bartonella throughout their large geographic range in Latin America [15,16,24,44]. Vampire bats are of particular concern because they subsist on blood, which could create opportunities for Bartonella transmission to humans and livestock either from bites during blood feeding or through vector sharing facilitated by close proximity [45–48]. Here, we capitalize on a two-year, spatially replicated study of vampire bats to examine the genetic diversity and infection prevalence of Bartonella, including its geographic structure across the vampire bat range as well as individual and temporal correlates of infection status. To explore possible transmission routes of this bacterial pathogen, we also test for associations between bat fly infestation and Bartonella infection status, which would support vector-borne transmission, and by screening bat saliva and fecal samples for evidence of Bartonella DNA, which would support transmission through bites or grooming and environmental exposure to bacteria shed in feces, respectively.

**Materials and methods**

**Vampire bat sampling**

Samples were collected as described in Becker et al. [49] in 2015 and 2016 across seven sites in Peru (Departments of Amazonas [AM], Cajamarca [CA], and Loreto [LR]) and two sites in Belize (Orange Walk [OW] District). We sampled sites one to two times annually, capturing one to 17 individuals per site and sampling interval (S1 Table). To screen for Bartonella by PCR, up to 30 μL blood was stored on Whatman FTA cards at room temperature. To assess the presence of Bartonella in saliva and feces, we collected oral and rectal swabs from vampire bats in Peru. Swabs were preserved in 2 mL RNalater (Invitrogen) at –80˚C until laboratory analyses. For Peru sites sampled in 2016, we also recorded the number of bat flies per vampire bat [32].

**Ethics statement**

Field procedures were approved by the University of Georgia Animal Care and Use Committee (A2014 04-016-Y3-A5) and the University of Glasgow School of Medical Veterinary and Life Sciences Research Ethics Committee (Ref08a/15); all procedures were conducted in accordance with accepted guidelines for humane wildlife research as outlined by the American Society of Mammalogists [50]. Bat capture, sample collection, and exportation were authorized by the Belize Forest Department under permits CD/60/3/15(21) and WL/1/1/16(17) and by the Peruvian Government under permits RD-009-2015-SERFOR-DGGSPFFS, RD-264-2015-SERFOR-DGGSPFFS, and RD-142-2015-SERFOR-DGGSPFFS. Access to genetic resources from Peru was granted under permit RD-054-2016-SERFOR-DGGSPFFS.

**Sequencing and phylogenetic analysis of vampire bat Bartonella**

We analyzed samples that were previously screened for the presence of Bartonella by Becker et al. [49] using nested PCR to amplify a region of the citrate synthase gene (gltA) [51]. Among the Bartonella-positive samples, we randomly selected 5–10 positive samples per site for Sanger sequencing (n = 51). PCR products were purified with DNA Clean & Concentrator Kits (Zymo Research) and sequenced in both directions at the Georgia Genomics Facility. Resulting chromatograms were checked for quality and trimmed using Geneious (Biomatters) [52]. Post-trimmed forward and reverse sequences were assembled to create 348 base pair (bp) consensus sequences for each sample (n = 35; the quality of 16 chromatograms was too low).
Sequences were considered part of the same genotype if they had >96% identity in gltA, an established cut-off for Bartonella species identification [53]. Sequences with >99.7% similarity were considered the same genetic variant [54]. We used a Chi-squared test with the p value generated via a Monte Carlo procedure with 1000 simulations [55] to assess whether our defined Bartonella genotypes were associated with region (i.e., Belize, eastern Peruvian Amazon, western Peruvian Amazon).

Two datasets were created for phylogenetic analyses. Dataset 1 was designed to assess the spatial structure of vampire bat–associated Bartonella across Latin America and therefore included our new sequences plus all previously reported gltA sequences from Desmodus rotundus. Dataset 2 was designed to capture the relatedness of the new sequences to all previously described Bartonella spp. regardless of isolation source, which comprised sequences generated in this study plus sequences obtained by conducting a BLAST search of each new sequence against GenBank, selecting the top 10 hits, and removing duplicates. For both datasets, consensus sequences were aligned using MAFFT. Phylogenetic analyses were carried out in MrBayes using the GTR+gamma model suggested by jModeltest2 [56]. For dataset 1 (Desmodus-associated sequences), we fit a codon partitioned substitution model by linking rates in codon positions 1 and 2 separately from codon position 3. For dataset 2, we used a simpler non-partitioned model because the more complex codon-partitioned model failed to converge. Dataset 2 included one sequence from Brucella abortus (Genbank Locus: MIJJ01000003.1) as an outgroup [13]. Both datasets were run for 2.5 million generations with convergence checked and burn-in periods selected by assessing posterior traces in Tracer [57]. With dataset 1, we analyzed spatial clustering of vampire bat Bartonella by country (Belize, Guatemala, Mexico, Peru) using Bayesian Tip Association Significance Testing (BaTS) [58]. We here selected 1,000 trees from the posterior distribution of the MrBayes run and compared the country-level clustering to a null distribution from 10,000 trees with swapped tip associations [58].

**Statistical analyses of Bartonella infection status**

We analyzed 193 samples from Desmodus rotundus to test whether temporal variation (season and year) and individual risk factors (e.g., age, sex) explain differences in Bartonella infection, using generalized mixed effects models (GLMMs) with binomial errors and a logit link fit with the lme4 package in R [59,60]. We fit a single GLMM with an interaction between site and year to first test if prevalence varied over years across sampling locations; we excluded one site from this analysis (i.e., LR6) owing to sampling in only 2015. We included bat identification number (ID) as a random effect to account for multiple sampling of a small number of bats (n = 6). To assess seasonality in infection, we fit a separate GLMM with season (spring, summer, fall) as a predictor to data from two sites in Peru (AM1 and CA1) sampled across seasons (n = 63). We also fit a generalized additive model (GAM) with restricted maximum likelihood, binomial response, and a cyclic cubic regression spline for Julian date using the mgcv package [61]. We randomly selected repeatedly sampled bats, as including bat ID as a random effect here overfit the GAM.

To identify individual risk factors for Bartonella infection, we fit a single GLMM with bat age, forearm size, sex, and reproductive status; we also included interactions between sex and reproduction, sex and age, sex and forearm size, and reproduction and forearm size. We included categorical livestock biomass as a predictor in the GLMM to control for a previously observed negative association with Bartonella infection (121/173 positive bats) [49]. We fit this GLMM to a reduced dataset free of missing values (n = 189), included bat ID nested within site as a random effect, and calculated marginal $R^2 (R^2_m)$ to assess model fit [62]. Finally, for a
data subset ($n = 40$ bats sampled in Peru in 2016), we fit two separate GLMs with bat fly intensity and presence as predictors to test whether ectoparasites explained \textit{Bartonella} infection status. We fit a separate GLM with quasi-Poisson errors to test for sex and age differences in bat fly intensity.

\textbf{Assessment of \textit{Bartonella} in saliva and feces}

To examine possible transmission of \textit{Bartonella} through biting, grooming, blood sharing, or fecal–oral exposure, we used metagenomic data from a parallel study to screen vampire bat saliva and fecal samples for \textit{Bartonella} DNA. Three saliva and three fecal pools were shotgun sequenced, each containing nucleic acid extractions from swabs collected from ten vampire bats from one to two colonies. Pooled samples contained individuals from the same colonies of bats tested for \textit{Bartonella} in blood through PCR, though not necessarily the same individuals.

As described previously [8], total nucleic acid was extracted from swabs and pooled equally according to RNA concentration. Pooled samples were DNase treated and ribosomal RNA depleted, then cDNA synthesis was performed. Libraries were prepared using a KAPA DNA Library Preparation Kit for Illumina (KAPA Biosystems) modified for low input samples, and were individually barcoded during the PCR reamplification step [10]. The libraries included in this study were combined in equimolar ratios with other metagenomic libraries for sequencing on an Illumina NextSeq500 at the University of Glasgow Centre for Virus Research.

Reads were demultiplexed according to barcode and quality filtered using TrimGalore [63,64] with a quality threshold of 25, minimum read length of 75 bp, and clipping the first 14 bp of the read. Low complexity reads were filtered out using the DUST method and PCR duplicates removed using PRINSEQ [65]. We screened cleaned reads for the \textit{Bartonella} genotypes detected in this study using nucleotide BLAST [66] against a custom database composed of the PCR-generated \textit{Bartonella} sequences from this study, retaining only the best alignment (the high-scoring segment pair with the lowest e-value) for a single query–subject pair. To investigate the presence of \textit{Bartonella} species other than genotypes detected in blood samples from vampire bats, cleaned reads were \textit{de novo} assembled into contigs using the assembly only function of SPAdes [67]. Individual reads and contigs were screened for sequences matching \textit{Bartonella} using protein alignment in Diamond [68], and close matches at the protein level were further characterized by nucleotide BLAST against the Genbank nt database. As the \textit{gltA} gene is not highly transcribed, we also tested sequences for matches to \textit{Bartonella} DNA-directed RNA polymerase subunit B (\textit{rpoB}). We selected two \textit{rpoB} sequences (Genbank accessions KY629892 and KY629911) from a study of vampire bat \textit{Bartonella} [16] for which the same individuals exhibited 100% identity in the \textit{gltA} gene to our blood sequences, and we used Bowtie2 to map quality filtered reads and contigs to those sequences [69].

Lastly, because nucleic acid pools were DNase treated for metagenomic sequencing, potentially reducing detection sensitivity, we used the same nested PCR protocol as used for blood-derived DNA [51] to test for the presence of \textit{gltA} in DNA from individual saliva and fecal swab samples that made up metagenomic pools ($n = 58$; 28 saliva and 30 feces). As with our blood samples, we randomly selected a subset of positive amplicons for Sanger sequencing.

\textbf{Results}

\textbf{Genetic diversity of vampire bat \textit{Bartonella}}

\textit{Bartonella} prevalence across the 193 vampire bats included in this study was 67\%. Our phylogenetic analysis of 35 vampire bat \textit{Bartonella} sequences showed 78.8–100\% pairwise identity in \textit{gltA} and revealed at least 11 paraphyletic genotypes (S2 Table). BaTS analysis of all \textit{Desmodus-
associated Bartonella showed significant phylogenetic clustering by country (association index = 3.81, parsimony score = 31.51, p<0.001), although most vampire bat Bartonella genotypes were still widely distributed (Fig 1). For the 11 genotypes delineated from our 35 sequences, we observed no association with the geographic study region (χ² = 23.3, p = 0.27). Genotypes 1 and 2 were detected across all regions, and genotypes 7–10 were detected within both Belize and Peru, highlighting the broad distribution of vampire bat Bartonella genotypes (Fig 2); however, genotype 3 was unique to both regions of Peru, genotypes 4–6 were unique to the western Peruvian Amazon, and genotype 11 was only detected in Belize.

We also assessed the phylogenetic position of our vampire bat Bartonella sequences among known Bartonella genotypes (Fig 3, S3 Table). Half of our Peruvian and Belizian sequences (18/35) were nearly identical (>99.7% identity) to Bartonella from common vampire bats (Desmodus rotundus) from Mexico (e.g., GenBank accession numbers KY629837 and MF467803), again confirming the wide geographic distribution of these genotypes. Other sequences (9/35) fell within the same clade (>96% pairwise identity) as Bartonella from bat flies (Streblia diacini) in Panama (JX416251), from Parnell’s mustached bat (Pteronotus pamelii) in Mexico (e.g., KY629828), from phytophagous bats in Peru (e.g., Carollia perspicillata; JQ071384) and Guatemala (e.g., Glossophaga soricina; HM597202), or from Mexican vampire bats as noted above. Eight sequences were novel (<96% identity to GenBank sequences) but were most similar to Bartonella from phytophagous bats in Costa Rica (e.g., 90–93% to KJ1816666 [Anoura geoffroyi]) and from Mexican vampire bats (e.g., 93% to MF467776).

Other novel sequences were weakly related to B. bovis from livestock in Israel and Malaysia (e.g., 89–90% to KJ909844 and KR733183), to B. chomelii from cattle in Spain (e.g., 89% to KM215693), to B. capreoli from elk in the United States (e.g., 89% to HM167503), and to B. schoenbuchensis from roe deer in Germany (e.g., 89% to AJ278186); indeed, posterior support for a bat–ruminant clade was low (<50%; Fig 3). Our BLAST procedure also identified weakly related Bartonella from rodents (e.g., 90% to Rattus norvegicus from the United States [KC763951] and 92% to Apodemus agrarius from China [KX549996]) and from carnivores (e.g., 89% to Procyon lotor from the United States [CP019786]). However, these livestock, rodent, and carnivore sequences formed separate phylogenetic clades from bat- and bat fly–derived Bartonella sequences (Fig 3). Despite the geographic proximity of our field sites to Brazil, our BLAST procedure found no Bartonella sequences similar to those recently described in Brazilian bat or rodent species [70–72]. An additional phylogenetic tree that includes these recently identified Bartonella is provided in S1 Fig.

Temporal patterns in infection

Bartonella was detected by PCR in all nine sites in each year, with prevalence ranging from 30–100% (Fig 4). Prevalence did not differ by year across all sites (χ² = 3.13, p = 0.54) nor within individual sites (site × year; χ² = 2.82, p = 0.90). The seasonality GLMM for the western Peruvian Amazon (n = 63) showed no difference in odds of infection between spring, summer, and fall (χ² = 1.99, p = 0.37; S2 Fig). The GAM also showed no significant seasonal variation (χ² = 0, p = 0.68; S2 Fig). Recaptures were rare (n = 6) but showed changes in infection from negative to positive (n = 2, 68–424 days) and from positive to negative (n = 2, 15–369 days; S3 Fig).

Individual risk factors for infection

After controlling for site-level livestock biomass, vampire bat sex and forearm size were the strongest predictors of infection (Fig 5); no interactions were significant (all χ² ≤ 1.18, p ≥ 0.28) and were dropped from the final GLMM (R²_m = 0.28). The odds of Bartonella infection were highest for vampire bats with larger forearms (OR = 1.2, p<0.001) and for males (OR = 5.41,
Fig 1. Phylogenetic relationships for the gltA gene among the sample of Bartonella genotypes detected in vampire bats from this study and vampire bat Bartonella genotypes from GenBank. All sequences are displayed with their GenBank accession numbers, and sequences from this study are listed in bold with bat ID numbers. The tips of all sequences are colored by geography, and diamonds depict posterior probabilities of nodes greater than 50%.

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were marginally higher for non-reproductive individuals (OR = 2.36, \( p = 0.10 \)), and
did not differ between subadult and adult bats (OR = 1.58, \( p = 0.38 \)); our sample did not con-
tain juveniles. Individual bat fly intensities were highly variable (0–28, median = 7.5) and
showed overdispersion (\( \phi = 5.08 \) in an intercept-only quasi-Poisson GLM). The bat fly GLMs
showed that neither ectoparasite intensity (OR = 0.98, \( p = 0.81 \)) nor ectoparasite presence
\( (\chi^2 = 1.13, p = 0.29) \) were associated with \textit{Bartonella} infection status. We note that the majority of
infected bats in this sample were infested with at least one bat fly (31/36), limiting conclusive
assessment of the ectoparasite–infection relationship. Our multivariable quasi-Poisson GLM
showed that ectoparasite load did not vary by bat sex \( (\chi^2 = 0.86, p = 0.35) \) or bat age \( (\chi^2 = 0.09, p = 0.77) \).

**Comparison of \textit{Bartonella} detected in blood, saliva, and feces**

There were no matches in any of the screened saliva and fecal metagenomic pools to the \textit{Barto-
nella gltA} sequences detected in the blood or to previously published \textit{Bartonella rpoB}
sequences. The saliva pool from Amazonas had no matching \textit{Bartonella}-like reads or contigs
(S4 Table), while one read each from the Loreto and Cajamarca saliva pool was assigned as
\textit{Bartonella} by nucleotide BLAST; however, these reported species assignments should be
Fig 3. Phylogenetic relationships for the gltA gene among the sample of Bartonella sequences detected in vampire bats and top BLAST hits from GenBank (S3 Table). Bartonella sequences from this study are displayed with genotype and bat ID numbers, and accession numbers. Sequences from GenBank are colored by host taxa and provided with accession numbers, species, and sampling location. Diamonds depict posterior probabilities of nodes greater than 50%.

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interpreted cautiously as they are based on one read and percent identity was low. Pooled fecal samples from all departments of Peru contained *Bartonella*-like reads and contigs. *Bartonella ancashensis*, *B. australis*, and *B. bacilliformis* were all identified at both the read and contig level in fecal samples. However, because percent identity was relatively low, species assignments should again be interpreted cautiously. Subsequent BLAST hits following the top hit also frequently (though not always) matched to *Bartonella*, suggesting the presence of poorly characterized *Bartonella* species present or that these may be matches to other bacteria.

In contrast, nested PCR of individual swabs detected *gltA* in 21.4% of saliva samples (6/28) and 30% of fecal samples (9/30). For swab samples that were also assessed by PCR in blood (n = 15 for saliva, n = 28 for feces), both corresponding positive saliva samples were positive in blood; most positive fecal samples were also PCR positive in blood, although one fecal-positive sample was PCR negative in blood (S4 Fig). For our random subset of sequenced positive saliva (n = 4) and fecal (n = 5) samples, phylogenetic analyses suggested that all sequences shared a minimum of 97% identity to one or more of our 35 blood-derived *Bartonella* sequences (S5 Table, Fig 6). In many cases, saliva and fecal sequences were the same genotype as blood sequences derived from the same geographic region (e.g., the saliva sequence from D234 shared >96% identity to the blood sequence from D98, both from AM1). For the one case in which we sequenced positive samples from the same individual bat (i.e., D203), both the blood sequence...
and fecal sequence shared 100% identity (S5 Table, Fig 6). For the few sequences at the lower range of our similarity spectrum, BLAST still demonstrated that the closest relatives were all derived from vampire bats (i.e., 8368 from CA1 was identical to MF467797 from Mexico).

**Discussion**

Despite an increasing focus on *Bartonella* genetic diversity and prevalence in bat communities, individual risk factors and transmission routes of this pathogen in bats remain largely unknown. For example, a survey of vampire bats within Guatemala found neither geographic, dietary, demographic, or viral coinfection correlates of *Bartonella* infection status [44]. Using a larger sample across a more diverse range of study sites and timepoints, we here show that *Bartonella* is genetically diverse, geographically widespread and endemic within vampire bat
populations, and that individual-level odds of infection are highest for large, male, and non-reproductive bats. Furthermore, we use several approaches to suggest vector-borne transmission to be likely in addition to possible direct contact and environmental sources of *Bartonella* exposure in bats.

The *Bartonella* genotypes we identified were paraphyletic and closely related to those from other vampire bat populations, other Neotropical bat species, or bat flies. Although BLAST also identified *Bartonella* spp. sequenced from rodents, carnivores, and livestock within our hit selection criteria, these consistently formed separate phylogenetic clades that did not contain bat- or bat fly–derived *Bartonella* (Fig 3). These phylogenetic patterns indicate that *Bartonella*...
has commonly shifted between bat host species in the Americas but do not support frequent transmission between bats and other host groups. Our BaTS analysis also showed that vampire bat Bartonella sequences clustered by country more than expected by chance. However, given that several Bartonella genotypes were present in vampire bats from both Central and South America, we suspect this clustering mostly resulted from variation in locally abundant genotypes rather than true barriers to the spread of Bartonella. Because vampire bats are largely sedentary and non-migratory [45], dispersal of these Bartonella genotypes across large distances is unlikely to be attributable to bat movement alone. Bartonella genotypes may also have infected vampire bats over long evolutionary timescales, and thus the biogeography of the pathogen may have followed that of its host. Alternatively, Bartonella dispersal by other arthropod vectors (e.g., ticks) or other bat species that share Bartonella genotypes with vampire bats may be conceivable and could be resolved by further field surveys combined with population genetic analyses of alternative bat host species, arthropod vector species, and Bartonella genotypes.

Few studies have examined temporal patterns of bat Bartonella, emphasizing the general need for more longitudinal studies to understand how pathogens persist in bat populations [1,6]. Here, Bartonella was detected at relatively high prevalence across both study years within each sampling site, and neither year nor its interaction with site were predictive within our analyses. Similarly, no temporal patterns in Bartonella were observed for a limited sample of Myotis mystacinus, Pipistrellus spp., Myotis daubentonii, and Nyctalus noctula in the United Kingdom [41]. Such findings contrast with highly seasonal Bartonella infections in rodents, which show high prevalence in summer and fall due to seasonality in birth and ectoparasite intensity [39,40]. The lack of seasonality in our western Peruvian Amazon sample in particular could simply be due to low statistical power; alternatively, no seasonality in infection could also be explained by the non-seasonal or less-pronounced birth pulses observed for vampire bats (but see [73]). While high Bartonella prevalence in bats has been proposed to stem from persistent infection [15], this seems unlikely, as we observed possible clearance of infection in some recaptured bats. While this could also reflect bacteria DNA loads too low to be detected by PCR, infection risk did not increase with age, as would also be expected if bats could not clear infection [74]. However, we do note that our sample only contained adult (n = 162) and subadult (n = 28) bats, limiting more robust tests of age-dependent infection. Alternatively, Bartonella infections could be chronic and vary in infection intensity over time or could become latent (i.e., be undetectable in erythrocytes but persist in endothelial cells), particularly as infection does not appear to confer long-term immunity [36]. Such explanations could be confronted in future work with larger sample sizes of recaptured bats, multiple assessments of infection status over time, and quantitative PCR.

Bat forearm size, sex, and reproductive status were important predictors of Bartonella infection status, with odds of infection being higher in larger, male, and non-reproductive bats. While subadult status itself was not an important predictor of Bartonella infection, these findings could suggest higher risk in young male bats that are relatively large for their age. Our previous work has shown stronger innate immune defense (i.e., bacterial killing ability) in reproductive (mostly male) vampire bats, also suggesting greater susceptibility of non-reproductive hosts [49]. Similarly, subadults across a Mexican bat community also had higher odds of Bartonella infection [16], and young male vampire bats play key roles in the long-distance dispersal of rabies virus [43] and display higher rates of rabies exposure, possibly owing to more direct contacts during the first year of life [75]. Larger forearm size could also relate to direct contact if larger bats are more dominant and aggressive, as found in other phyllostomid bat species [76].

Although vector-borne transmission is generally assumed for Bartonella in other hosts [12,19,25,77], including some Neotropical bats [16,78], infection status in vampire bats was
not associated with bat fly intensity. Further supporting this observation, male bats had higher odds of Bartonella infection but did not differ in their bat fly intensities compared to females. Weak correspondence between bat fly intensity at the time of sampling and Bartonella infection thus may cast doubt on bat flies as a primary transmission route. Time lags could provide one reason for this discrepancy, given that new Bartonella infections may take days or weeks to develop and become detectable and over which time ectoparasite load may have changed due to the mobile nature of bat flies [26,36,79]. On the other hand, it is possible that vector presence (rather than abundance) is a more important driver of transmission. Unfortunately, nearly all bats in this study had ectoparasites, so comparisons of Bartonella presence in bats with and without bat flies had little statistical power (31/36 Bartonella-positive bats were infested with at least one bat fly). Given that ectoparasitism predicted Bartonella infection more generally across a Mexican bat community [16], larger sample sizes with greater variation in bat fly intensity could provide better inference. However, our phylogenetic analysis does provide a tentative line of evidence supporting vector-borne transmission, as several of Bartonella genotypes fell within the same clade as Bartonella from streblid bat flies [29]. A recent survey of Mexican bats and their sympatric bat flies suggested that corresponding hosts and their bat flies had varied Bartonella genotypes, although one vampire bat did show complete sequence homology with the Bartonella from its paired bat flies [37]. As genetic similarity between Bartonella in bat flies and hosts has been interpreted as evidence of vector-borne transmission in other bat species [29,54], further assessments of Bartonella genotypes between vampire bats and their various ectoparasites (bat flies but also ticks) would shed additional light on possible routes of vector-borne transmission.

Lastly, we analyzed bat saliva and feces using metagenomics and PCR to explore alternative transmission routes, namely through close contact and fecal exposure. Metagenomics detected no Bartonella DNA matching to glnA or rpoB in either saliva or fecal pools. This absence could be explained in that the short sequences (345–425 bp) used as targets, and the large size of bacterial genomes together make the likelihood of detecting a specific gene low. However, the Bartonella-like reads and contigs recovered from saliva and feces were short fragments (51–258 bp) and showed low homology to known Bartonella from GenBank (S4 Table). Notably, we used a similar approach to search for other bacteria (i.e., hemoplasmas) and found clear evidence of their presence [8]. While this could suggest true absence of Bartonella from bat saliva and feces, our PCR found Bartonella in a subset of individual saliva and fecal samples. This discrepancy between methods could stem from treating saliva and fecal pools with DNase before metagenomic sequencing. Furthermore, phylogenetic analyses confirmed that these sequences were closely related to those identified in blood, which argues against these PCR positives only representing bacteria derived from environmental contamination or from feeding on prey. PCR results further showed strong correspondence between blood and saliva, suggesting that Bartonella infection may be systemic in vampire bats. While fecal and blood PCR results also mostly matched, we found one case where a bat was negative in blood but positive in feces. As consumption of ectoparasites during grooming has been observed in other bat families (e.g., Pteropodidae [80]), this discrepancy could suggest the incidental ingestion of ectoparasites during grooming and that this does not lead to systemic infection more generally indicated by the concordance between blood, saliva, and fecal positives and their close genetic similarity.

Similar prevalence of Bartonella in saliva and feces suggests that direct contact and environmental exposure could serve as complementary transmission routes to arthropod vectors. The presence of Bartonella in saliva samples contrasts with previous work showing an absence of Bartonella in vampire bat saliva [44,81], providing evidence for possible direct transmission. Bartonella in fecal samples could also suggest environmental transmission between bats [18]. Both saliva-borne and fecal–oral transmission of vampire bat Bartonella could further pose
potential risks to humans or livestock, either through bites during feeding or by environmental exposure of humans that enter roosts or to domestic animals exposed to bat feces [18,38,48,82]. For the former pathway, however, a recent survey of Bartonella in Mexican ruminants did not identify being bitten by vampire bats as a risk factor for infection [81], and our phylogenetic results provide relatively more support for the possibility of vector-borne transmission. Vector-borne transmission of vampire bat Bartonella might reduce their potential to infect humans or livestock, given the high host specificity of most bat flies [31,32]. However, ectoparasite transfer between individuals could still occur during pupal deposition and close contact [83], facilitating Bartonella transmission within vampire bat colonies and to other bat species. While our analyses of ectoparasitism only considered bat flies, we have observed heavy tick burdens of vampire bats in other field sites (e.g., Belize). Bartonella has been detected in ticks infesting other bats [28], and these ectoparasites could also be more likely to facilitate cross-species transmission [30]. Metagenomics also potentially identified Bartonella anchashensis and B. bacilliformis in vampire bat fecal samples, and these species cause notable infectious disease in humans likely through phlebotomine sand flies in Andean regions of Peru [84,85]. Controlled infection trials and more extensive phylogenetic analyses of Bartonella in vampire bats, their various ectoparasites, and sympatric prey are therefore needed to examine the contributions of different transmission routes for bacterial spread within vampire bats and to recipient prey and to confirm whether saliva and feces represent viable transmission routes. Given the high rates of bat bites and proximity to wildlife, humans, and domestic animals that define vampire bat ecology, such efforts to verify the possibility and frequency of oral and environmental exposures would elucidate Bartonella transmission dynamics in this common host species and the risks of cross-species transmission.

Supporting information

S1 Table. Sample size, sampling dates, and Bartonella prevalence in vampire bats.

S2 Table. Pairwise similarity (%) between the 35 vampire bat–associated Bartonella sequences from this study, calculated with Kimura’s 2-parameters distance model. Sequences with greater than 96% similarity were assumed to be part of the same genotype for downstream analyses.

S3 Table. Pairwise similarity (%) between each vampire bat Bartonella genotype from this study and the top 10 BLAST hits from GenBank.

S4 Table. Bartonella-like reads and contigs detected in pooled saliva and fecal samples using metagenomics. Department describes where samples in a pool were taken. Bartonella-like reads were identified by Diamond protein blast, and read matches are a list of those that were identified as Bartonella based on nucleotide blast. Bartonella-like contigs were identified by Diamond protein blast, and contig matches are a list of those that were identified as Bartonella based on nucleotide blast. Length is contig length in base pairs, and K-mer coverage is the depth of coverage for the largest k-value used in the SPAdes assembly.

S5 Table. Pairwise similarity (%) between the 35 vampire bat–associated Bartonella sequences from this study and the nine sequences derived from a subset of positive saliva (n = 4) and fecal (n = 5) samples, calculated with Kimura’s 2-parameters distance model.
Sequences with greater than 96% similarity were assumed to be part of the same *Bartonella* genotype.

**S1 Fig.** Phylogenetic relationships for the *gltA* gene among the sample of *Bartonella* sequences detected in vampire bats, top BLAST hits from GenBank (S3 Table), and sequences from recent studies of bat and rodent *Bartonella* from Brazil. *Bartonella* sequences from our study are listed with genotype, bat ID numbers, and accession numbers. Sequences from GenBank are colored by host taxa and provided with accession numbers, host species, and sampling location; red tips display recent *gltA* sequences from Brazilian bats and rodents.

**S2 Fig.** Lack of seasonality in vampire bat *Bartonella* from western Peruvian Amazon sites where colonies were sampled multiple times in each of the two years. Modeled prevalence (black line) and 95% confidence intervals (grey) from the GLMM (left; *n* = 63) and GAM (right; *n* = 61). Data points are jittered and colored by infection status (black = positive, white = negative).

**S3 Fig.** Dynamics of *Bartonella* infection status for recaptured vampire bats (*n* = 6) during 2015 and 2016. Infected bats are shown in red, uninfected bats are shown in black.

**S4 Fig.** Counts of positive and negative *Bartonella* PCR results for saliva (A) and fecal samples (B) for which blood was also assessed for evidence of infection.

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References
1. Hayman DTS, Bowen RA, Cryan PM, McCracken GF, O’Shea TJ, Peel AJ, et al. Ecology of Zoonotic Infectious Diseases in Bats: Current Knowledge and Future Directions. Zoonoses Public Health. 2013; 60: 2–21. https://doi.org/10.1111/zph.12000 PMID: 22958281
2. Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev. 2006; 19: 531–545. https://doi.org/10.1128/CMR.00017-06 PMID: 16847084
3. de Thoisy B, Bourhy H, Delaval M, Pontier D, Dacheux L, Darcissac E, et al. Biocological Drivers of Rabies Virus Circulation in a Neotropical Bat Community. PLoS Negl Trop Dis. 2016; 10: e0004378. https://doi.org/10.1371/journal.pntd.0004378 PMID: 26808820
4. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. The natural history of Hendra and Nipah viruses. Microbes Infect. 2001; 3: 307–314. https://doi.org/10.1016/S1286-4579(01)01384-3 PMID: 11334748
5. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005; 310: 676–679. https://doi.org/10.1126/science.1118391 PMID: 16195424
6. Olival KJ, Hayman DTS. Filoviruses in Bats: Current Knowledge and Future Directions. Viruses. 2014; 6: 1759–1788. https://doi.org/10.3390/v6041759 PMID: 24747773
7. Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM. Detection of pathogenic Leptospira spp. infections among mammals captured in the Peruvian Amazon basin region. Am J Trop Med Hyg. 2000; 63: 255–258. PMID: 11421373
8. Volokhov DV, Becker DJ, Bergner LM, Camus MS, Orton RJ, Chizhikov VE, et al. Novel hemotropic mycoplasmas are widespread and genetically diverse in vampire bats. Epidemiol Infect. 2017
9. Mühl dorfer K. Bats and Bacterial Pathogens: A Review. Zoonoses Public Health. 2013; 60: 93–103. https://doi.org/10.1111/j.1863-2378.2012.01536.x PMID: 22862791
10. Maillard R, Petit E, Chomel B, Lacroux C, Schelcher F, Vayssier-Taussat M, et al. Endocarditis in Cattle Caused by Bartonella bovis. Emerg Infect Dis. 2007; 13: 1383–1385. https://doi.org/10.3201/eid1309.070236 PMID: 18252116
11. Kosoy M, Bai Y, Sheff K, Morway C, Baggett H, Maloney SA, et al. Identification of Bartonella infections in febrile human patients from Thailand and their potential animal reservoirs. Am J Trop Med Hyg. 2010; 82: 1140–1145. https://doi.org/10.4269/ajtmh.2010.09-0778 PMID: 20519614
12. Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, et al. Bartonella spp. in Fruit Bats and Blood-Feeding Ectoparasites in Madagascar. PLOS Negl Trop Dis. 2015; 9: e0003532. https://doi.org/10.1371/journal.pntd.0003532 PMID: 25706653
13. Bai Y, Hayman DTS, McKee CD, Kosoy MY. 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. 2015; 9: e0003478. https://doi.org/10.1371/journal.pntd.0003478 PMID: 25635826
14. Kosoy M, Bai Y, Lynch T, Kuzmin IV, Nieszgodz M, Franka R, et al. Bartonella spp. in bats, Kenya. Emerg Infect Dis. 2010; 16: 1875–1881. https://doi.org/10.3201/eid1612.100601 PMID: 21122216
15. Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, Turmelle A, et al. Bartonella spp. in bats, Guatemala. Emerg Infect Dis. 2011; 17: 1269–1272. https://doi.org/10.3201/eid1707.101867 PMID: 21762584
16. Stuckey MJ, Chomel BB, Galvez-Romero G, Olave-Leyva J, Obregón-Morales C, Moreno-Sandoval H, et al. Bartonella Infection in Hematophagous, Insectivorous, and Phytophagous Bat Populations of Central Mexico and the Yucatan Peninsula. Am J Trop Med Hyg. 2017; 97: 413–422. https://doi.org/10.4269/ajtmh.16-0680 PMID: 28722567
17. Han H-J, Wen H, Zhao L, Liu J, Luo L-M, Zhou C-M, et al. Novel Bartonella Species in Insectivorous Bats, Northern China. PLOS ONE. 2017; 12: e0167915. https://doi.org/10.1371/journal.pone.0167915 PMID: 28608122
18. Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT. Bats as Reservoir Hosts of Human Bacterial Pathogen, *Bartonella mayotimonensis*. Emerg Infect Dis. 2014; 20: 960–967. https://doi.org/10.3201/ eid2006.130956 PMID: 2485623

19. Lilley TM, Wilson CA, Bernard RF, Willcox EV, Vesterinen EJ, Webber QMR, et al. Molecular Detection of Candidatus *Bartonella mayotimonensis* in North American Bats. Vector-Borne Zoonotic Dis. 2017; 17: 243–246. https://doi.org/10.1089/vbz.2016.2080 PMID: 28165925

20. Stuckey MJ, Boulouis H-J, Clignet F, Picard-Meyer E, Servat A, Aréchiga-Ceballos N, et al. Potentially Zoonotic *Bartonella* in Bats from France and Spain. Emerg Infect Dis. 2017; 23: 539–541. https://doi.org/10.3201/eid2303.160934 PMID: 28221109

21. Lin EY, Tsigris C, Baddour LM, Lepidi H, Rolain J-M, Patel R, et al. Candidatus *Bartonella mayotimonensis* and endocarditis. Emerg Infect Dis. 2010; 16: 500. https://doi.org/10.3201/eid1603.081673 PMID: 20202430

22. Plowright RK, Parrish CR, McCallum H, Hudson PJ, Ko AI, Graham AL, et al. Pathways to zoonotic spillover. Nat Rev Microbiol. 2017; 15: 502–510. https://doi.org/10.1038/nrmicro.2017.45 PMID: 28555733

23. Wood JLN, Leach M, Waldman L, MacGregor H, Fooks AR, Jones KE, et al. A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. Philos Trans R Soc B Biol Sci. 2012; 367: 2881–2892. https://doi.org/10.1098/rstb.2012.0228 PMID: 22966143

24. Morse SF, Olival KJ, Kosoy M, Billeter S, Patterson BD, Dick CW, et al. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Strebilidae, Nycteribiidae). Infect Genet Evol. 2012; 12: 1717–1723. https://doi.org/10.1016/j.meegid.2012.06.009 PMID: 22771358

25. Gill JS, Roweley WA, Bush PJ, Viner JP, Gilchrist MJR. Detection of human blood in the bat tick *Carios* (Ornithodoros) *kelleyi* (Acari: Argasidae) in Iowa. J Med Entomol. 2004; 41: 1195–1196. PMID: 15605658

26. Billeter SA, Levy MG, Che mol BB, Breitschwerdt EB. Vector transmission of *Bartonella* species with emphasis on the potential for tick transmission. Med Vet Entomol. 2008; 22: 1–15. https://doi.org/10.1111/j.1365-2915.2008.00713.x PMID: 18380649

27. Billeter SA, Hayman DTS, Peel AJ, Baker K, Wood JLN, Cunningham A, et al. *Bartonella* species in bat flies (Diptera: N ycteribiidae) from western Africa. Parasitology. 2012; 139: 324–329. https://doi.org/10.1017/S00311820110 02113 PMID: 22309510

28. Loftis AD, Gill JS, Schri ever ME, Levin ML, Emer reeva ME, Gilchrist MR, et al. Detection of *Rickettsia, Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). J Med Entomol. 2005; 42: 473–480. PMID: 15962801

29. Morse SF, Olival KJ, Kosoy M, Billeter S, Patterson BD, Dick CW, et al. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Strebilidae, Nycteribiidae). Infect Genet Evol. 2012; 12: 1717–1723. https://doi.org/10.1016/j.meegid.2012.06.009 PMID: 22771358

30. Gill JS, Roweley WA, Bush PJ, Viner JP, Gilchrist MJR. Detection of human blood in the bat tick *Carios* (Ornithodoros) *kelleyi* (Acari: Argasidae) in Iowa. J Med Entomol. 2004; 41: 1179–1181. PMID: 15605658

31. Dick CW. High host specificity of obligate ectoparasites. Ecol Entomol. 2007; 32: 446–450. https://doi.org/10.1111/j.1365-2311.2007.00836.x

32. Ter Hofste de HM, Fenton MB, Whitaker JO. Host and host-site specificity of bat flies (Diptera: Strebilidae and Nycteribiidae) on Neotropical bats (Chiroptera). Can J Zool. 2004; 82: 616–626.

33. Mannerings AO, Osikowicz LM, Restif O, Nyarko E, Suu-Ire R, Cunningham AA, et al. Exposure to Bat-associated *Bartonella* spp. among Humans and Other Animals, Ghana. Emerg Infect Dis. 2016; 22: 922–924. https://doi.org/10.3201/eid2205.151908 PMID: 27088812

34. Duncan AW, Maggi RG, Breitschwerdt EB. *Bartonella* DNA in dog saliva. Emerg Infect Dis. 2007; 13: 1948. https://doi.org/10.3201/eid1312.070653 PMID: 18258056

35. Kim Y, Seo K, Lee J, Choi E, Lee H, Hwang C, et al. Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in cats and dogs in Korea. J Vet Sci. 2009; 10: 85–87. https://doi.org/10.4142/jvs.2009.10.1.85 PMID: 19255530

36. Breitschwerdt EB, Kordick DL. *Bartonella* infection in animals: carri ership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000; 13: 428–438. PMID: 10885985

37. Moskaluk AE, Stuckey MJ, Jaffe DA, Kasten RW, Aguilar-Setién A, Olave-Leyva JI, et al. Molecular Detection of *Bartonella* Species in Blood-Feeding Bat Flies from Mexico. Vector-Borne Zoonotic Dis. 2018; 18: 258–265. https://doi.org/10.1089/vbz.2017.2213 PMID: 29652641

38. O’Shea TJ, Cryan PM, Hayman DTS, Plowright RK, Streicker DG. Multiple mortality events in bats: a global review. Mammal Rev. 2016; 46: 175–190. https://doi.org/10.1111/mam.12064 PMID: 29755179
39. Kosoy M, Mandel E, Green D, Marston E, Childs J. Prospective Studies of *Bartonella* of Rodents. Part I. Demographic and Temporal Patterns in Population Dynamics. Vector-Borne Zoonotic Dis. 2004; 4: 285–295. https://doi.org/10.1089/vbz.2004.4.285 PMID: 15671735

40. Morway C, Kosoy M, Eisen R, Montenieri J, Sheff K, Reynolds PJ, et al. A longitudinal study of *Bartonella* infection in populations of woodrats and their fleas. J Vector Ecol. 2008; 33: 353–364. PMID: 19263856

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

42. McKee CD, Hayman DT, Kosoy MY, Webb CT. Phylogenetic and geographic patterns of *Bartonella* host shifts among bat species. Infect Genet Evol. 2016; 44: 382–394. https://doi.org/10.1016/j.meegid.2016.07.033 PMID: 27473781

43. Streicker DG, Winternitz JC, Satterfield DA, Condori-Condori RE, Broos A, Tello C, et al. Host–pathogen evolutionary signatures reveal dynamics and future invasions of vampire bat rabies. Proc Natl Acad Sci. 2016; 113: 10926–10931. https://doi.org/10.1073/pnas.1605871113 PMID: 27621441

44. Wray AK, Olival KJ, Morán D, Lopez MR, Alvarez D, Navarrete-Macias I, et al. Viral Diversity, Prey Preference, and *Bartonella* Prevalence in Desmodus rotundus in Guatemala. EcoHealth. 2016; https://doi.org/10.1007/s10393-016-1183-z PMID: 27660213

45. Greenhal AM, Schmidt U. Natural history of vampire bats. CRC Press, Inc.; 1988.

46. Bobrowiec PED, Lemes MR, Gribel R. Prey preference of the common vampire bat (*Desmodus rotundus*, Chiroptera) using molecular analysis. J Mammal. 2015; 96: 54–63. https://doi.org/10.1093/jmammal/gyu002

47. Hoare CA. Vampire bats as vectors and hosts of equine and bovine trypanosomes. Acta Trop. 1965; 22: 204. PMID: 4379528

48. Streicker DG, Allgeier JE. Foraging choices of vampire bats in diverse landscapes: potential implications for land-use change and disease transmission. J Appl Ecol. 2016; 53: 1280–1288. https://doi.org/10.1111/1365-2664.12690 PMID: 27499553

49. Becker DJ, Czirjak GA, Volokhov DV, Bentz AB, Carrera JE, Camus MS, et al. Livestock abundance predicts vampire bat demography, immune profiles and bacterial infection risk. Phil Trans R Soc B. 2018; 373: 20170089. https://doi.org/10.1098/rstb.2017.0089 PMID: 29531144

50. Sikes RS, Care A. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J Mammal. 2016; 97: 663–688. https://doi.org/10.1093/jmammal/gyw078 PMID: 29692469

51. Bai Y, Gilbert A, Fox K, Osikowicz L, Kosoy M. *Bartonella rochalimae* and *B. vinsonii* subsp. berkoffii in wild carnivores from Colorado, USA. J Wildl Dis. 2016; 52: 844–849. https://doi.org/10.7589/2016-01-015 PMID: 27529290

52. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199 PMID: 22543367

53. Scola BL, Zeaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the Bartonella paradigm. Trends Microbiol. 2003; 11: 318–321. https://doi.org/10.1016/S0966-842X(03)00143-4 PMID: 12875815

54. Judson SD, Frank HK, Hadly EA. Bartonellae are Prevalent and Diverse in Costa Rican Bats and Bat Flies. Zoonoses Public Health. 2015; 62: 609–617. https://doi.org/10.1111/zph.12188 PMID: 25810119

55. Hope ACA. The Simplified Monte Carlo Significance Test Procedure. J R Stat Soc Ser B Methodol. 1968; 30: 582–598.

56. Wood S. Generalized additive models: an introduction with R. CRC press; 2006.
62. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol. 2013; 4: 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

63. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet journal. 2011; 17: 10–12. https://doi.org/10.14806/ej.17.1.200

64. Andrews S. FastQC: a quality control tool for high throughput sequence data. 2010.

65. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011; 27: 863–864. https://doi.org/10.1093/bioinformatics/btr026 PMID: 21278185

66. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215: 403–410. https://doi.org/10.1016/S0022-2836(90)80360-3 PMID: 2231712

67. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012; 19: 455–477. https://doi.org/10.1089/cmb.2012.0021 PMID: 22506599

68. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015; 12: 59–60. https://doi.org/10.1038/nmeth.3176 PMID: 25402007

69. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 2009; 10: R25. https://doi.org/10.1186/gb-2009-10-3-r25 PMID: 19261174

70. Ikeda P, Seki MC, Carrasco AOT, Rudiak LV, Miranda JMD, Gonçalves SMM, et al. Evidence and molecular characterization of Bartonella spp. and hemoplasmas in neotropical bats in Brazil. Epidemiol Infect. 2017; 1–15.

71. Sousa KCM de, Amaral RB do, Herrera HM, Santos FM, Macedo GC, Pinto PCE de A, et al. Genetic Diversity of Bartonella spp. in Wild Mammals and Ectoparasites in Brazilian Pantanal. Microb Ecol. 2018; 1–11. https://doi.org/10.1007/s00248-017-1136-0 PMID: 29313064

72. Gonçalves LR, de Mendonça Favacho AR, Roque ALR, Pacheco V, et al. Association of Bartonella species with wild and synanthropic rodents in different Brazilian biomes. Appl Environ Microbiol. 2016; 82: 7154–7164. https://doi.org/10.1128/AEM.02447-16 PMID: 27736785

73. Delpietro HA, Russo RG, Carter GG, Lord RD, Delpietro GL. Reproductive seasonality, sex ratio and philopatry in Argentina’s common vampire bats. R Soc Open Sci. 2017; 4: 160959. https://doi.org/10.1098/rsos.160959 PMID: 28484615

74. Anh PH, Van Cuong N, Son NT, Tue NT, Kosoy M, Woolhouse MEJ, et al. Diversity of Bartonella spp. in Bats, Southern Vietnam. Emerg Infect Dis. 2015; 21: 1266–1267. https://doi.org/10.3201/eid2107.141760 PMID: 26079810

75. Streicker DG, Recuenco S, Valderrama W, Benavides JG, Vargas I, Pacheco V, et al. Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. Proc R Soc B. 2012; 279: 3384–3392. https://doi.org/10.1098/rspb.2012.0538 PMID: 22696521

76. Ortega J, Guerrero JA, Maldonado JE. Aggression and Tolerance by Dominant Males of Artibeus jamaicensis: Strategies to Maximize Fitness in Harem Groups. J Mammal. 2008; 89: 1372–1378. https://doi.org/10.1644/08-MAMM-S-056.1

77. Stuckey MJ, Chomel BB, de Fleurieu EC, Aguilar-Setien A, Boulouis H-J, Chang C. Stenotus australis RV, Andrianarimisa A, Raselimana AP, Goodman SM. Rates of hematophagy and bugs: A review. Comp Immunol Microbiol Infect Dis. 2017; 55: 20–29. https://doi.org/10.1016/j.cimid.2017.09.001 PMID: 29127990

78. Ramanantsalama RV, Andriananirainina A, Rasiellamanana AP, Goodman SM. Bartonella species, bats and bugs: A review. Comp Immunol Microbiol Infect Dis. 2017; 55: 20–29. https://doi.org/10.1016/j.cimid.2017.09.001 PMID: 29127990

79. Olival KJ, Dittmar K, Bai Y, Rostal MK, Lei BR, Daszak P, et al. Bartonella in vampire bats. J Wildl Dis. 2015; 51: 274–278. https://doi.org/10.7589/2014-04-113 PMID: 25390361

80. Dick CW, Patterson BD. Against all odds: explaining high host specificity in dispersal-prone parasites. Int J Parasitol. 2007; 37: 871–876. https://doi.org/10.1016/j.ijpara.2007.02.004 PMID: 17382332

81. Bartonella in vampire bats. J Trop Ecol. 2007; 23: 177–189.
84. Minnick MF, Anderson BE, Lima A, Battisti JM, Lawyer PG, Birtles RJ. Oroya Fever and Verruga Peruana: Bartonelloses Unique to South America. PLoS Negl Trop Dis. 2014; 8: e2919. https://doi.org/10.1371/journal.pntd.0002919 PMID: 25032975

85. Mullins KE, Hang J, Clifford RJ, Ommus-Leone F, Yang Y, Jiang J, et al. Whole-Genome Analysis of Bartonella ancashensis, a Novel Pathogen Causing Verruga Peruana, Rural Ancash Region, Peru. Emerg Infect Dis. 2017; 23: 430–438. https://doi.org/10.3201/eid2303.161476 PMID: 28221130

86. Becker RA, Wilks AR. Maps in S. ATbackslash T Bell Lab Stat Res Rep 932. 1993; Available: http://euler.stat.yale.edu/Courses/1997-98/200fall97/FAQ/Maps.in.S.pdf