Bartonella spp. in a Puerto Rican Bat Community

Author(s): Kevin J. Olival, Katharina Dittmar, Ying Bai, Melinda K. Rostal, Bonnie R. Lei, Peter Daszak, and Michael Kosoy
Source: Journal of Wildlife Diseases, 51(1):274-278.
Published By: Wildlife Disease Association
DOI: http://dx.doi.org/10.7589/2014-04-113
URL: http://www.bioone.org/doi/full/10.7589/2014-04-113

DOI: 10.7589/2014-04-113

Journal of Wildlife Diseases, 51(1), 2015, pp. 274–278

Bartonella spp. in a Puerto Rican Bat Community

Kevin J. Olival,[1,5] Katharina Dittmar,[2] Ying Bai,[3] Melinda K. Rostal,[1] Bonnie R. Lei,[1,4] Peter Daszak,[1] and Michael Kosoy[3] [1]EcoHealth Alliance, 460 W 34th Street, New York, New York 10001, USA; [2]Department of Biological Sciences, State University of New York at Buffalo, 109 Cooke Hall, Buffalo, New York 14260, USA; [3]Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, 3156 Rampart Road, Fort Collins, Colorado 80521, USA; [4]Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138, USA; [5]Corresponding author (email: olival@ecohealthalliance.org)

ABSTRACT: We captured and sampled 68 bats of six species from a shared roosting site in Puerto Rico in April 2012. Bats were screened for *Bartonella* spp. by culture and confirmed by PCR and sequencing for the *gltA* gene. Bartonella cultures were obtained from blood specimens of 9/51 (18%) individuals from three species (*Artibeus jamaicensis*, *Brachyphylla cavernarum*, and *Monophyllus redmani*). Phylogenetic analysis of the *gltA* sequences showed that *M. redmani* was infected with multiple, diverse *Bartonella* strains, and *A. jamaicensis* was infected with a strain related to a strain from a congeneric host. Ectoparasite load could possibly explain observed differences in *Bartonella* diversity and prevalence between bat species in this community, and we suggest future research to substantiate these preliminary findings.

Key words: Bacteria, *Bartonella*, bat, *Chiroptera*, diversity, host range, Puerto Rico, vector.

Bartonella spp. (order *Rhizobiales*) are diverse, intracellular alpha-proteobacteria that can cause persistent bacteremia in reservoir host species (Harms and Dehio 2012). Bartonellae are known to infect a wide-range of mammalian wildlife species globally, including more than 20 putative new *Bartonella* spp. identified from bats in Kenya, Guatemala, Peru, and Thailand (Kosoy et al. 2010; Bai et al. 2011, 2012). Half of all described *Bartonella* spp. are zoonotic (Maggi et al. 2012), and although there is no evidence for bat-borne *Bartonella* transmission to humans, this may be possible given indirect exposure to vectors from synanthropic bat species or direct contact with other species (e.g., vampire bats [*Desmodus rotundus*]). Bartonellae are primarily transmitted by arthropod vectors, including fleas (Insecta: Siphonaptera), ticks (Arachnida: Parasitiformes), mites (Arachnida: Mesostigmata), and flies (Insecta: Diptera) (Bai and Kosoy 2012). Ectoparasitic bat flies in the families Nycteribiidae and Streblidae are likely important vectors of, and possibly reservoirs for, bartonellae within the order Chiroptera (Billeter et al. 2012; Morse et al. 2012).

Bartonella spp. have varying degrees of host specificity, and the reasons for this remain unclear but may be due to host phylogeny, vector host specificity, or spatial overlap of host species (Vayssier-Taussat et al. 2009). Globally, Lei and Olival (2014) found a statistically significant pattern of coevolution between *Bartonella* and their bat hosts. In arthropod vectors, *Bartonella* host specificity is largely unexplored, and sampling has been insufficient relative to their diversity. Some *Bartonella* spp. have a wide potential host and vector range (e.g., *Bartonella grahamii* has been detected in several flea species from rodents (Rodentia) and shrews (Soricidae; Hsieh et al. 2010). In bat flies, host specificity varies (Patterson et al. 2009) and vector-host relationships may possibly determine the phylogenetic patterns observed in bat bartonellae. The degree of physical contact within shared habitat and host and vector population sizes may also influence *Bartonella* diversification and host range.

To test whether bats sharing the same roosting habitat (i.e., a cave) share similar *Bartonella* spp. genotypes, we captured and sampled individuals from a community of bats in Mata de Platano Nature Reserve in Puerto Rico from 10 to 13 April 2012, with a focus on Cueva de los Culebrones ("Cave of the Boas"; 18°24′N, 66°43′W). Cueva de los Culebrones houses a year-round

TABLE 1. Bat specimens examined from Cueva de los Culebrones, Puerto Rico, 10–13 April 2012. Bat species, number of individuals sampled, number for which *Bartonella* culture was attempted excluding the contaminated samples, number positive with (percent prevalence), and number of unique *Bartonella* genotypes for 379 base pair of the *gltA* gene and associated GenBank Accession numbers. The 95% confidence intervals (CIs) were calculated by multiplying the standard error by sample size–specific *t*-scores (*t*-distribution necessary given the small sample sizes).

Species	No. bats sampled	No. cultured	No. positive (%)	95% CI[a]	No. genotypes (GenBank accession)
Artibeus jamaicensis	4	4	1 (25)	0–94	1 (KJ530741)
Brachyphylla cavernarum	2	1	1 (100)	n/a	1 (KJ530742)
Erophylla sezekorni	17	14	0	0–20	
Monophyllus redmani	20	9	7 (78)	46–100	4 (KJ530743–KJ530746)
Mormoops blainvillei	21	19	0	0–15	
Pteronotus quadridens	4	4	0	0–60	
Total	68	51	9 (18)	7–25	

[a] n/a = not applicable.

occupancy of >300,000 bats, including species from three families (Phyllostomidae, Mormoopidae, Noctilionidae; Rodríguez-Durán 1998). Bats roost and coroost in distinct patterns across the cave's length and thermal gradient (28–40 C), although admixture zones within the cave facilitate direct contact of species (Dittmar et al. 2011). We captured bats using a custom-made, 4-m^2 harp trap set outside of the cave between 1700 hours and 2100 hours. Additionally, four *Artibeus jamaicensis* were captured from a colony (ca.15 individuals) roosting alone in a small cave within 500 m of Cueva de los Culebrones. We aimed to capture 15 individuals of each species to have 95% confidence in detecting bartonellae given an assumed prevalence of ≥20%; three species were sampled above the desired sample size, and three species were not (Table 1).

Captured bats were held individually in cloth bags and nonlethally sampled after approved protocols (IACUC G2011-106 Tufts University). Between 6 and 228 μL (mean, 40 μL) of blood (maximum 10% of blood volume) was collected from the brachial vein using previously described methods (Smith et al. 2010). Whole blood was diluted in the field with sterile phosphate-buffered saline at a ratio of 1:5 and frozen directly in liquid nitrogen. Fecal swabs, urine, and oropharyngeal swabs were also collected from each individual for a separate study. Bats were identified to species in the field following Gannon et al. (2005), and sex, reproductive status, forearm length, weight, and body condition score were recorded. Ectoparasites were opportunistically collected from ~20% of individuals (12 randomly selected bats proportionally distributed across species), and a 3-mm wing biopsy was taken from each bat for genetic species confirmation. Ectoparasite vouchers are stored in the Dittmar laboratory at State University of New York, Buffalo, New York, USA; no bat voucher specimens were collected. Bat species identifications were confirmed at the American Museum of Natural History's Sackler Institute for Comparative Genomics, New York, New York, USA, by sequencing ~450 base pairs (bp) of the *cytb* gene.

Bartonella was cultured from diluted blood at the Centers for Disease Control and Prevention (CDC) Bartonella Laboratory in Fort Collins, Colorado, USA, using the methods of Bai et al. (2012). All *Bartonella* cultures were frozen at −80 C and stored in the facility at CDC for long-term vouchering. Total genomic DNA was extracted from blood according to the blood protocol of QIAamp DNA Mini Kit (Qiagen, Valencia California, USA) and

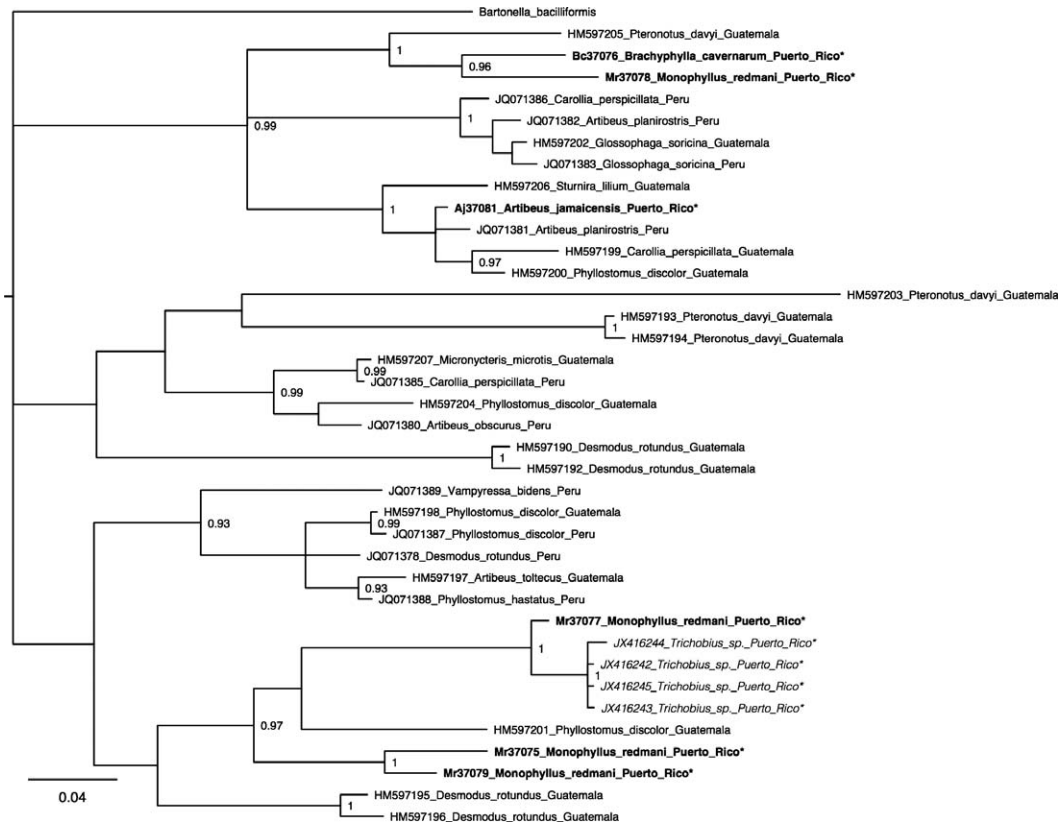

FIGURE 1. Bayesian phylogeny of *Bartonella* genotypes from Puerto Rican bats (bold, with asterisk) from Cueva de los Culebrones, Puerto Rico, 10–13 April 2012, including published genotypes from Puerto Rico bat flies (italic, with asterisk), and all other unique *Bartonella* genotypes previously published from New World bats. Based on 379 base pairs of *gltA* gene. GTR+I+G substitution model, with 10,000,000 generations, and posterior probability values >0.90 shown for each node.

screened by PCR for *Bartonella* using previously published primers (BhCS781.p and BhCS1137.n) to amplify a 379-bp region of the citrate synthase–encoding gene (*gltA*; Bai et al. 2012). All products were sequenced in both directions using an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). The *Bartonella gltA* sequences obtained from Puerto Rican bats were aligned with previously reported *Bartonella* genotypes originating from New World bats, *Trichobius* sp. from Cueva de los Culebrones (Morse et al. 2012); *Bartonella bacilliformis* was used as an outgroup using default parameters in MUSCLE (Edgar 2004). Sequences were trimmed to 338 bp, and a Bayesian phylogeny was generated using MrBayes 3.2 (Ronquist et al. 2012). We utilized a GTR+I+G substitution model, with 10,000,000 generations, sampling every 5000th generation with four heated chains and a burn in length of 2,000,000. Final standard deviation of split frequencies was <0.01.

Bartonella was cultured from 9/51 (18%) bats examined (16 blood samples contaminated during the first week of culture were excluded). Six bat species were captured and sampled. Positive species were *A. jamaicensis*, *Brachyphylla cavernarum*, and *Monophyllus redmani*, all in the family Phyllostomidae (Table 1). *Monophyllus redmani* had the highest prevalence (77%) and was infected by

four unique genotypes of bartonellae (Table 1 and Fig. 1). No bartonellae were detected in *Erophylla sezekorni*, *Mormoops blainvillei*, and *Pteronotus quadridens*, despite relatively large samples sizes for some species, (e.g., 17 *E. sezekorni* and 21 *M. blainvillei*). We cannot exclude the possibility that these species are completely *Bartonella*-free; however, we have 95% confidence that they have a prevalence lower than 20% at this site using the methods described.

Bartonella isolates from Puerto Rican bats were diverse (six unique genotypes, Table 1). Nucleotide identity among the Puerto Rico *Bartonella gltA* sequences ranged from 99.7% to 81.7% (7–60 raw nucleotide differences) with a mean identity of 86.8%. Individuals of *M. redmani* had the greatest diversity with four genotypes (Fig. 1); one genotype clustered together with a *Bartonella* obtained from *Trichobius* spp. bat flies previously collected from the cave wall at this same site (Morse et al. 2012). Two other *Bartonella* genotypes from *M. redmani* form part of a larger clade that includes bartonellae from bat flies from the same site (97.4% nucleotide identity) and *Phyllostomus discolor* from Guatemala (Fig. 1). We also found an isolate from *A. jamaicensis* grouping in a well-supported clade with a congeneric host from Peru, *Artibeus planirostris* (Fig. 1).

While the sample sizes in this study were relatively small and not equal across all species, we observed one species of bat, *M. redmani*, to be infected with multiple genotypes of *Bartonella*, including strains found in *Trichobius* spp. flies that commonly parasitize this host. We notably did not observe any sharing of *Bartonella* genotypes between bat species known to share the same ectoparasite species but did observe *M. redmani* shared a *Bartonella* genotype with *B. cavernarum* from the same cave (Fig. 1). We found different *Bartonella* prevalences between host species in this bat community that could possibly be driven by vector specificity and parasite load, although our limited sample sizes precluded testing of this. *Monophyllus redmani* harbors the highest ectoparasite load in the cave, with 4.00 ± 1.15 per individual (Krichbaum et al. 2009), and had the highest prevalence and diversity of bartonellae. Similarly, *M. blainvillei*, though well sampled, was not found positive for bartonellae and is reported in the literature as having the lowest diversity of ectoparasites and no streblid bat flies (Kurta et al. 2007). Future studies and better sampling are needed to assess the role that ectoparasites play in bat *Bartonella* transmission and to test further the patterns of *Bartonella* host range and diversification we explored here. This should include exhaustive sampling of bat parasites to quantify parasite load and *Bartonella* infection rates, and experimental studies using captive bats and ectoparasites with known infection status.

We thank Solon Morse and Krishna Balasubramaniam for field assistance. Funding provided in part by a National Institutes of Health National Institute of Allergy and Infectious Diseases nonbiodefense Award (1R01 AI079231-01), the Centers for Disease Control and Prevention's Global Disease Detection Program, and the US Agency for International Development's Emerging Pandemic Threats Program PREDICT project (GHN-A-OO-09-00010-00).

LITERATURE CITED

Bai Y, Kosoy M. 2012. Bartonella infections in rodents and bats in tropics. In: *Current topics in tropical medicine*, Rodriguez-Morales A, editor. InTech, Rijeka, Croatia, pp. 51–66.

Bai Y, Kosoy M, Recuenco S, Alvarez D, Moranv D, Turmelle A, Ellison J, Garcia DL, Estevez A, Lindblade K, et al. 2011. *Bartonella* spp. in bats, Guatemala. *Emerg Infect Dis* 17:1269–1272.

Bai Y, Recuenco S, Gilbert AT, Osikowicz LM, Gomez J, Rupprecht C, Kosoy MY. 2012. Prevalence and diversity of *Bartonella* spp. in bats in Peru. *Am J Trop Med Hyg* 87:518–523.

Billeter SA, Hayman DT, Peel A, Baker K, Wood JL, Cunningham AA, Suu-Ire R, Dittmar K, Kosoy M. 2012. Bartonella species in bat flies (Diptera:

Nycteribiidae) from western Africa. *Parasitology* 139:324–329.

Dittmar K, Morse S, Gruwell M, Mayberry J, Diblasi E. 2011. Spatial and temporal complexities of reproductive behavior and sex ratios: A case from parasitic insects. *PLoS One* 6:e19438.

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.

Gannon MR, Rodriguez-Duran A, Kurta A. 2005. *Bats of Puerto Rico: An island focus and a Caribbean perspective*. Texas Tech University Press, Lubbock, Texas.

Harms A, Dehio C. 2012. Intruders below the radar: Molecular pathogenesis of *Bartonella* spp. *Clin Microbiol Rev* 25:42–78.

Hsieh JW, Tung KC, Chen WC, Lin JW, Chien LJ, Hsu YM, Wang HC, Chomel BB, Chang CC. 2010. Epidemiology of *Bartonella* infection in rodents and shrews in Taiwan. *Zoonoses Public Health* 57:439–446.

Kosoy M, Bai Y, Lynch T, Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Breiman RF, Rupprecht CE. 2010. *Bartonella* spp. in bats, Kenya. *Emerg Infect Dis* 16:1875–1881.

Krichbaum K, Perkins S, Gannon MR. 2009. Host-parasite interactions of tropical bats in Puerto Rico. *Acta Chiropt* 11:157–162.

Kurta A, Whitaker JO, Wrenn WJ, Soto-Centeno JA. 2007. Ectoparasitic assemblages on Mormoopid bats (Chiroptera: Mormoopidae) in Puerto Rico. *J Med Entomol* 44:953–958.

Lei BR, Olival KJ. 2014. Contrasting patterns in mammal–bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. *PLoS Negl Trop Dis* 8:e2738.

Maggi RG, Harms CA, Breitschwerdt EB. 2012. Bartonellosis: An emerging disease of humans, domestic animals, and wildlife. In: *New directions in conservation medicine: Applied cases of ecological health*, Aguirre A, Ostfeld R, Daszak P, editors. Oxford University Press, Oxford, UK, pp. 239–256.

Morse SF, Olival KJ, Kosoy M, Billeter SA, Patterson BD, Dick CW, Dittmar K. 2012. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). *Infect Genet Evol* 12:1717–1723.

Patterson BD, Dick CW, Dittmar K. 2009. Nested distributions of bat flies (Diptera: Streblidae) on Neotropical bats: Artifact and specificity in host-parasite studies. *Ecography* 32:481–487.

Rodríguez-Durán A. 1998. Distribution and nonrandom aggregations of the cave bats of Puerto Rico. *J Mammal* 79:141–146.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542.

Smith CS, De Jong C, Field H. 2010. Sampling small quantities of blood from microbats. *Acta Chiropt* 12:255–258.

Vayssier-Taussat M, Le Rhun D, Bonnet S, Cotte V. 2009. Insights in *Bartonella* host specificity. *Ann N Y Acad Sci* 1166:127–132.

Submitted for publication 28 April 2014.
Accepted 27 August 2014.