Bartonella Infection in Hematophagous, Insectivorous, and Phytophagous Bat Populations of Central Mexico and the Yucatan Peninsula

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Abstract. Although emerging nonviral pathogens remain relatively understudied in bat populations, there is an increasing focus on identifying bat-associated bartonellae around the world. Many novel *Bartonella* strains have been described from both bats and their arthropod ectoparasites, including *Bartonella mayotimonensis*, a zoonotic agent of human endocarditis. This cross-sectional study was designed to describe novel *Bartonella* strains isolated from bats sampled in Mexico and evaluate factors potentially associated with infection. A total of 238 bats belonging to seven genera were captured in five states of Central Mexico and the Yucatan Peninsula. Animals were screened by bacterial culture from whole blood and/or polymerase chain reaction of DNA extracted from heart tissue or blood. *Bartonella* spp. were isolated or detected in 54 (22.7%) bats, consisting of 41 (38%) hematophagous, 10 (16.4%) insectivorous, and three (4.3%) phytophagous individuals. This study also identified *Balantiopteryx plicata* as another possible bat reservoir of *Bartonella*. Univariate and multivariate logistic regression models suggested that *Bartonella* infection was positively associated with blood-feeding diet and ectoparasite burden. Phylogenetic analysis identified a number of genetic variants across hematophagous, phytophagous, and insectivorous bats that are unique from described bat-borne *Bartonella* species. However, these strains were closely related to those bartonellae previously identified in bat species from Latin America.

INTRODUCTION

Bartonellae are gram-negative, primarily vector-borne bacteria that colonize the endothelial and red blood cells of numerous mammalian hosts, including carnivores, ruminants, rodents, and bats.¹ Many *Bartonella* species are also recognized as zoonotic, potentially impacting the health of nonhuman animals and people.² Those responsible for human illness are associated with a broad spectrum of clinical symptoms, including culture-negative endocarditis and prolonged fever of unknown origin.²

Novel *Bartonella* variants continue to be described from bats and their arthropod ectoparasites sampled across the globe.³ Although there is an increasing focus on identifying these bat-associated bartonellae, emerging bacterial pathogens remain relatively understudied and poorly understood in bat populations.⁴ The current data on *Bartonella* infection in bats suggest a high diversity of genetic variants, though little is known about those epidemiologic factors influencing transmission.

The primary objective of this cross-sectional study was to further describe novel *Bartonella* variants in bat hosts while also evaluating factors potentially associated with infection. Mexico itself has a rich chiropteran diversity with 134 species, including the northernmost range of the common vampire bat, *Desmodus rotundus*.⁴ A limited number of studies have been conducted about the presence of *Bartonella* spp. in potential terrestrial wildlife reservoirs in Mexico,^{5–7} and further investigation is needed, especially in volant mammals.

MATERIALS AND METHODS

Study sites and sampling methods. A total of 238 bats representing seven genera (*Artibeus*, *Balantiopteryx*, *Carollia*, *Desmodus*, *Glossophaga*, *Pteronotus*, and *Sturmira*) were captured in Central Mexico and the Yucatan Peninsula between September 2011 and December 2013. Sampling efforts spanned 14 sites across five Mexican states, which included three locations in Morelos, one in Hidalgo, one in Veracruz, six in Jalisco, and three in Yucatan (Figure 1). Bats were collected using hand or mist nets placed near agricultural corridors, cave entrances, building structures, and livestock. Ninety-five bats were sampled in Morelos, 61 in Jalisco, 45 in Yucatan, 28 in Hidalgo, and nine in Veracruz. After collecting visible ectoparasites, bats were rehydrated orally with a sugar or saline solution. Animals were then anesthetized by administration of ketamine hydrochloride (5 mg/kg) and medetomidine hydrochloride (50 µg/kg) via intramuscular injection. Animals were subsequently measured, weighed, aged, sexed, and identified to species using a dichotomous key by field specialists. Based on relevant ecological dietary groupings, as previously described,⁸ all bats were discretely categorized as hematophagous, insectivorous, or phytophagous (inclusive of frugivorous and nectarivorous species).

Bats were finally exsanguinated via intracardiac puncture, drawing between 10 µL and 1.5 mL of whole blood depending on size of the bat species. Necropsies were performed to collect organ fragments of heart, liver, spleen, and brain for laboratory diagnostics. Whole blood and organ samples were initially preserved in the field using liquid nitrogen or dry ice before being transferred to –80°C freezers at the Hospital de Pediatría, Centro Médico Nacional Siglo XXI, IMSS in Mexico City. All diagnostic tests were performed at the Veterinary Public Health Laboratory at the University of California, Davis.

Ethics statement. All animals were chemically restrained and humanely treated until euthanasia end points, in accordance

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FIGURE 1. Coordinates of bats sampled in the present study, which include 14 sites across five states: three locations in Morelos, one in Hidalgo, one in Veracruz, six in Jalisco, and three in Yucatan. Red squares represent sampling conducted in 2011, green triangles 2012, and blue circles 2013. Spatial maps of sampling locations were created in R using the ggplot2 package (<http://ggplot2.org>). This figure appears in color at www.ajtmh.org.

with the Institutional Animal Care and Use Committee (IACUC Protocol 17164) at the University of California, Davis. Scientific collection permits were issued by the Mexico Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA License FAUT – 0250) and the Veterinary and Zootechnical School at the National University of Mexico (UNAM Permit B00.02.02/1026 1708). Importation permits were issued by the U.S. Department of Health and Human Services (PHS No. 2011-07-090, 2013-08-085, 2014-07-130).

Diagnostic tests. *Bartonella* culturing techniques were adapted from previously described protocols.^{9,10} Whole blood up to 200 μ L was added at a 1:1 ratio to a mixture of M199 growth media, supplemented with 5% amphotericin B fungicide.^{11,12} This was plated across two heart infusion agar plates containing 5% rabbit blood and incubated in 5% CO₂ at 35°C for up to 7 weeks. *Bartonella* colonies were identified morphologically and a single colony from each sample was streaked onto a secondary blood agar plate for subculture. Counts were manually performed on original mix agar plates and colony-forming units (CFUs) were calculated as number per 100 μ L of whole blood.

After approximately 1 week, single-colony subcultures were harvested and subjected to DNA extraction. A suspension of bacterial colonies and tris buffer was heated for 15 minutes at 100°C, followed by a 10- to 15-second vortexing. After 5 minutes

of cooling at room temperature, the samples were centrifuged at 12,500 rpm at 4°C for 10 minutes. One hundred microliters of supernatant were then transferred into a sterile 1.5 mL vial containing 100 μ L of 2 $\times$ TE buffer. A working solution diluted to 1:10 was made by adding 5 μ L of the stock into a new 1.5 vial containing 45 μ L of TE buffer. DNA was separately extracted from 25 mg of heart tissue and whole blood when organs were not available using Qiagen DNeasy Blood and Tissue Kits (QIAGEN GmbH, Hilden, Germany) under the manufacturer's recommendations.²

Bartonella isolates were verified by amplification of a fragment of the citrate synthase gene (*gltA*) via polymerase chain reaction (PCR) with primers BhCS.781p and BhCS1137.n, as previously described (Table 1).^{9,13} DNA extracted from bat blood and heart tissue was similarly screened by PCR targeting *gltA* with primers CSH1f and BhCS1137.n.¹⁴ Amplicons were verified by gel electrophoresis and then cleaned using Qiagen QIAquick PCR Purification Kits. Samples were submitted for sequencing performed by the UC DNA Sequencing Facility in the College of Biological Sciences at the University of California, Davis. Those isolates verified as *Bartonella* spp. based on partial sequencing of *gltA* were further described at additional loci (*ftsZ*, *rpoB*, and 16S rRNA).^{15–17} All amplified products of *gltA*, *rpoB*, *ftsZ*, and 16S rRNA were sequenced in both directions and the obtained alignments were referenced against known *Bartonella* spp. deposited in

TABLE 1
Oligonucleotide primer pairs used in polymerase chain reaction amplifications for the detection of *Bartonella* DNA in bat samples

Primer pairs	5'-primer sequence-3'	Oligo orientation	Target locus
BhCS781.p	GGGGACCAGCTCATGGTGG	Forward	<i>gltA</i>
BhCS1137.n	AATGCAAAAAGAACAGTAAACA	Reverse	
CSH1f	GCGAATGAAGCGTGCCTAAA	Forward	<i>gltA</i>
BhCS1137.n	AATGCAAAAAGAACAGTAAACA	Reverse	
prAPT0244	GATGTGCATCCTACGCATTATGG	Forward	<i>rpoB</i>
prAPT0245	AATGGTGCCTCAGCAGGTATAAG	Reverse	
prAPT0257	GCCTTCAAGGAGTTGATTTTGTGTTGCCAAT	Forward	<i>ftsZ</i>
prAPT0258	ACGACCCATTTTCATGCATAACAGAAC	Reverse	
fD1	AGAGTTTGATCCTGGCTCAG	Forward	16S rRNA
rP2	ACGGCTACCTTGTTACGACTT	Reverse	

TABLE 2
Descriptive characteristics of bat species sampled in Mexico and screened for *Bartonella* infection

Dietary guild	Bat species	Number screened	<i>Bartonella</i> + culture and/or PCR (%)	Number of male (%)	Number of adults (%)
Hematophagous	<i>Desmodus rotundus</i>	108	41 (38)	70 (64.8)	60 (55.6)
Phytophagous	<i>Artibeus jamaicensis</i>	19	1 (5.2)	7 (36.8)	18 (94.7)
	<i>Artibeus lituratus</i>	40	0	13 (32.5)	37 (92.5)
	<i>Artibeus phaeotis</i>	1	0	1 (100)	1 (100)
	<i>Carollia</i> sp.	1	0	0	1 (100)
	<i>Glossophaga</i> spp.	3	0	1 (33.3)	3 (100)
	<i>Sturnira</i> spp.	5	2 (40)	1 (20)	4 (80)
Insectivorous	<i>Balantiopteryx plicata</i>	31	2 (6.5)	8 (25.8)	20 (64.5)
	<i>Pteronotus parnellii</i>	30	8 (26.7)	7 (23.3)	30 (100)
Totals		238	54 (22.7)	108 (45.4)	174 (73.1)

PCR = polymerase chain reaction.

TABLE 3

Bats positive for *Bartonella* spp. by culture and/or PCR of heart tissue, with associated GenBank accession numbers for *gltA*, *rpoB*, *ftsZ*, and 16S rRNA sequence fragments where available

Study ID number	Heart tissue <i>gltA</i>	Isolate <i>gltA</i>	Isolate <i>rpoB</i>	Isolate <i>ftsZ</i>	Isolate 16S rRNA
A01	NA	NA	NA	NA	NA
A02	NA	KY629869	KY629927	KY629826	KY635946
A03	NA	KY629868	KY629926	KY629825	KY635945
A05	NA	KY629834	KY629925	KY629824	KY635944
A07	NA	KY629837	KY629924	KY629823	KY635943
A08	KY629843	KY629863	KY629923	KY629822	KY635942
A09	NA	KY629862	KY629922	KY629821	KY635941
A10	KY629829	NA	NA	NA	NA
A14	KY629842	KY629840	KY629921	KY629820	KY635940
A15	KY629852	NA	NA	NA	NA
A17	NA	KY629867	KY629920	KY629819	KY635939
A18	KY629845	KY629866	KY629919	KY629818	KY635938
A19	NA	KY629874	KY629918	KY629817	KY635937
A20	NA	KY629860	KY629917	KY629816	KY635936
A21	NA	KY629871	KY629916	KY629815	KY635935
A22	KY629844	KY629864	KY629915	KY629814	KY635934
A23	NA	KY629859	KY629914	KY629813	KY635933
A24	NA	KY629861	KY629913	KY629812	KY635932
A25	NA	KY629877	KY629912	KY629811	KY635931
A27	KY629847	KY629878	KY629911	KY629810	KY635930
C02	NA	KY629839	KY629910	KY629809	KY635929
C07	NA	KY629858	KY629909	KY629808	KY635928
C10	NA	NA	NA	NA	NA
C14	KY629880	NA	NA	NA	NA
D01	NA	KY629855	KY629908	KY629807	KY635927
D04	NA	KY629854	KY629907	KY629806	KY635926
DR02	NA	KY629836	KY629906	KY629805	KY635925
DR03	NA	KY629865	KY629905	KY629804	KY635924
DR13	NA	KY629879	KY629904	KY629803	KY635923
DR15	NA	KY629870	KY629903	KY629802	KY635922
DR17	KY629848	NA	NA	NA	NA
DR18	NA	KY629873	KY629902	KY629801	KY635921
DR20	NA	KY629875	NA	NA	NA
DR22	NA	KY629857	KY629901	KY629800	KY635920
DR24	NA	KY629851	KY629900	KY629799	KY635919
DR28	NA	KY629876	KY629899	KY629798	KY635918
E01	KY629830	NA	NA	NA	NA
E03	KY629828	NA	NA	NA	NA
F02	NA	KY629884	KY629898	KY629797	KY635917
F09	NA	KY629882	KY629897	KY629796	KY635916
F10	NA	KY629881	KY629896	KY629795	KY635915
F13	NA	KY629883	NA	NA	NA
F14	NA	KY629885	KY629895	KY629794	KY635914
F24	NA	KY629856	NA	NA	NA
J03	NA	KY629850	KY629894	KY629793	KY635913
M01	NA	KY629853	KY629893	KY629792	KY635912
M09	NA	KY629849	KY629892	KY629791	KY635911
M11	NA	KY629838	KY629891	KY629790	KY635910
M12	NA	KY629835	KY629890	KY629789	KY635909
M13	KY629846	KY629832	KY629889	KY629788	KY635908
M16	KY629827	KY629841	KY629888	KY629787	KY635907
M25	NA	KY629831	KY629887	KY629786	KY635906
M35	NA	KY629872	KY629886	KY629785	KY635905
M42	NA	KY629833	NA	NA	NA

TABLE 4

Univariate analysis of factors associated with *Bartonella* infection in Mexican bats via logistic regression, with binary outcome (culture and/or PCR)

Variable	Category	Number (%) of <i>Bartonella</i> positive	Odds ratio	95% CI	χ^2 P value
Diet	Phytophagous	3 (4.3)	Reference		
	Insectivorous	10 (16.4)	4.31	1.13–16.49	0.033
	Hematophagous	41 (38)	13.46	3.97–45.61	< 0.0001
Sex	Female	26 (20)	Reference		
	Male	28 (25.9)	1.4	0.76–2.57	0.278
Age	Adult	29 (16.7)	Reference		
	Juvenile	25 (39.1)	3.21	1.69–6.09	0.0004
Ectoparasites			1.12	1.07–1.18	< 0.0001

CI = confidence interval; PCR = polymerase chain reaction.

GenBank/EMBL/DDBJ databases using BLAST. Initial phylogenetic trees were constructed from *gltA* sequences using the MrBayes plugin within Geneious version 8.1.7 using a 1,100,000 Markov Chain Monte Carlo and 100,000 burn-in length.^{18,19} Similarly, the concatenated phylogenetic tree of *Bartonella* isolates was based on sequences of all four described loci (*ftsZ*, *gltA*, *rpoB*, and 16S rRNA). To determine the taxonomic classification of the bat-associated *Bartonella* strains, the criteria previously proposed by Scola and others, in which the *gltA* and the *rpoB* gene sequences from each isolate were compared with those of validated published *Bartonella* species or subspecies.²⁰

Statistical analysis. Binomial proportion confidence intervals (CIs) for *Bartonella* prevalence data were calculated using Wald and Fisher's exact methods depending on sample size.²¹ Recorded variables, including bat dietary guild (categorical), sex and age (dichotomous), and ectoparasite burden (continuous) were initially screened using univariate logistic regression to check for statistically significant associations with infection status (dichotomous) by culture and/or PCR. Certain variables were reclassified in biologically meaningful groups, such as bat dietary guild, prior to univariate analysis, irrespective of sample size considerations.

Using forward stepwise selection methods, variables were retained in a multivariate logistic regression model for *Bartonella* infection determined by culture and/or PCR. Variables that induced changes greater than 10% in the log odds ratio (OR) of covariates after inclusion in the model were considered to be potential confounders, and evaluated along with current knowledge and biological plausibility.²² All logistic regression models and resulting adjusted disease ORs with 95% CI were generated in SAS, University Edition (SAS Institute Inc., Cary, NC) using two-sided testing and an alpha of 0.05.

RESULTS

The 238 sampled bats were composed of 108 (45.4%) hematophagous (*D. rotundus*), 69 (29%) phytophagous (*Artibeus*

jamaicensis, *Artibeus lituratus*, *Artibeus phaeotis*, *Carollia* spp., *Glossophaga* spp., and *Sturmira* spp.), and 61 (25.6%) insectivorous (*Balantiopteryx plicata* and *Pteronotus parnellii*) species (Table 2). Across all dietary groups, 108 (45.4%) bats were male and 174 (73.1%) adult.

Fifty-four bats (22.7%) were positive for *Bartonella* spp. by either culture or PCR (Table 3). Sequences for 52 of these 54 bats were deposited in GenBank (accession numbers shown in Table 3). Of those *Bartonella*-positive bats, 40 (16.8%) were only culture positive, seven (2.9%) were only PCR positive from heart tissue or blood, and another seven (2.9%) were both culture and PCR positive. *Bartonella*-positive bats belonged to the genera *Desmodus*, *Artibeus*, *Sturmira*, *Pteronotus*, and *Balantiopteryx*. This is the first report of the presence of *Bartonella* in bats of the genus *Balantiopteryx*, where two (6.5%) of the 31 bats tested positive (Table 2). Looking further at agreement between diagnostic tests, 138 (73%) bats were negative for *Bartonella* spp. by both culture and PCR. A simple kappa coefficient of 0.16 (95% CI: 0.03–0.30) increased marginally to 0.29 when only evaluating agreement between PCR and highly bacteremic versus low bacteremic culture positives (where high bacteremia was defined as more than 1,000 CFU/100 μ L blood).

Across dietary guilds, 41 (38%) hematophagous, 10 (16.4%) insectivorous, and three (4.3%) phytophagous bats were positive for *Bartonella* spp. by either culture or PCR. Univariate association between the three diet categories indicated that the odds of blood-feeding bats being infected with *Bartonella* were 13.46 (95% CI: 3.97–45.61) times those of phytophagous bats (Table 4). Further, the odds of insectivorous bats being *Bartonella* positive were 4.31 (95% CI: 1.13–16.49) times those of phytophagous species. Sex was not identified as statistically significant, as the odds of male bats being *Bartonella* positive were 1.4 (95% CI: 0.76–2.57) times greater than females. However, a one-unit increase in ectoparasite load on a bat host was associated with a 12% increase in the odds of *Bartonella* infection (OR: 1.12, 95% CI: 1.07–1.18).

TABLE 5

Multivariate analysis of factors associated with *Bartonella* infection in Mexican bats via logistic regression, with binary outcome (culture and/or PCR)

Variable	Category	Coefficient estimate	Adjusted odds ratio	95% CI	χ^2 P value
Diet	Phytophagous	Reference			
	Insectivorous	1.41	4.09	1.06–15.72	0.041
	Hematophagous	2.03	7.58	2.0–28.77	0.003
Age	Adult	Reference			
	Juvenile	0.58	1.79	0.86–3.73	0.123
Ectoparasites		0.06	1.06	1.003–1.12	0.037

CI = confidence interval; PCR = polymerase chain reaction.

FIGURE 2. Citrate synthase (*glTA*) sequences of bat-associated *Bartonella* variants detected in this study in relation to named species. Hematophagous bats are labeled in red, insectivorous bats in blue, phytophagous bats in green, and known *Bartonella* spp. in black with accession numbers. This figure appears in color at www.ajtmh.org.

The multivariate logistic regression model contained independent terms for diet, age, and total number of ectoparasites present (Table 5). Blood-feeding bats had 7.58 (95% CI: 2.0–28.77) times higher odds of *Bartonella*

infection as compared with phytophagous species. Odds of being *Bartonella* positive in insectivorous bats were 4.09 (95% CI: 1.06–15.72) times higher than phytophagous bats. Juveniles had 1.79 times higher odds for *Bartonella* infection

FIGURE 3. Citrate synthase (*gltA*) sequences of bat-associated *Bartonella* variants detected in this study in relation to those previously identified in bats from Latin America. Hematophagous bats from Mexico are labeled in red, insectivorous in blue, and phytophagous in green with sample type. *Bartonella* variants described from other studies are labeled in black with countries and accession numbers. This figure appears in color at www.ajtmh.org.

than adults, and this association became nonstatistically significant (95% CI: 0.86–3.73) after adjusting for diet and ectoparasites in the multivariate model. Increasing ectoparasite load by one bat fly was associated with a 6% increase in the odds of *Bartonella* infection in the host (OR: 1.06, 95% CI: 1.003–1.12).

Phylogenetic analysis identified *Bartonella* strains across hematophagous, phytophagous, and insectivorous bats that are unique from previously described species (Figure 2). However, the strains clustered with those bartonellae previously identified in bats from Latin America, including

Guatemala, Puerto Rico, Costa Rica, and Peru (Figure 3).^{23–26} Concatenated sequences of *ftsZ*, *gltA*, *rpoB*, and 16S rRNA from Mexican bat isolates provided further support in relation to the bat-associated *Bartonella* strains isolated in Finland, Taiwan, and Africa (Figure 4).^{17,27,28}

DISCUSSION

The number of studies on pathogens in bats is rapidly growing, with broad impacts that range from biodiversity conservation to public health. As bats and their arthropod

In addition to describing novel *Bartonella* strains, this study estimated the prevalence of *Bartonella* spp. in various bat populations from Mexico and identified factors associated with infection that can be targeted for further investigation. Although our cross-sectional design limits the direct

Blood-feeding bats had the highest odds of *Bartonella* infection, even after adjusting for age and ectoparasite burden.

TABLE 6
Comparison of *Bartonella* prevalence in bats sampled from different Latin American countries

Dietary guild	Bat genus/species	Mexico (this study)	Peru ²³	Guatemala ²⁶	French Guiana ³⁷	Puerto Rico ²⁵	Costa Rica ²⁴	Saint Kitts ³⁰	Total
Hematophagous	<i>Desmodus rotundus</i>	41/108 (38%)	10/18 (55.6%)	15/31 (48.4%)	NA	NA	0/1 (0%)	NA	66/159 (41.5%)
	<i>Diphylla ecaudata</i>	NA	0/1 (0%)	NA	NA	NA	NA	NA	NA
Insectivorous	<i>Pteronotus</i> sp.	8/30 (26.7%)	NA	7/10 (70%)	5/14 (35.7%)	0/4 (0%)	0/3 (0%)	NA	44/222 (19.8%)
	<i>Myotis</i> sp.	NA	1/6 (16.7%)	0/3 (0%)	NA	NA	3/4 (75%)	NA	NA
	<i>Balanopteryx plicata</i>	2/31 (6.5%)	NA	NA	NA	NA	NA	NA	NA
	<i>Micronycteris</i> sp.	NA	NA	1/3 (33.3%)	NA	NA	1/1 (100%)	NA	NA
	<i>Phyllostomus</i> sp.	NA	3/4 (75%)	8/9 (89%)	0/1 (0%)	NA	1/4 (25%)	NA	NA
	<i>Mollosus</i> sp.	NA	0/10 (0%)	NA	0/2 (0%)	NA	NA	0/27 (0%)	NA
	<i>Mormoops blainvillei</i>	NA	NA	NA	NA	0/19 (0%)	NA	NA	NA
	<i>Eumops auripendulus</i>	NA	NA	NA	0/10 (0%)	NA	NA	NA	NA
	<i>Noctilio albigentris</i>	NA	NA	NA	2/22 (9%)	NA	NA	NA	NA
	<i>Eptesicus brasiliensis</i>	NA	NA	NA	NA	NA	0/1 (0%)	NA	NA
	<i>Anoura geoffroyi</i>	NA	NA	NA	NA	NA	2/4 (50%)	NA	NA
	<i>Artibeus</i> sp.	1/60 (1.7%)	3/29 (10.3%)	1/17 (5.9%)	0/8 (0%)	1/4 (25%)	4/8 (50%)	18/34 (53%)	88/371 (23.7%)
	<i>Carollia</i> sp.	0/1 (0%)	6/33 (18.2%)	4/15 (26.7%)	0/2 (0%)	NA	5/15 (33.3%)	NA	NA
	<i>Sturnira</i> sp.	2/5 (40%)	1/1 (100%)	1/14 (7.1%)	NA	NA	3/10 (30%)	NA	NA
	<i>Glossophaga</i> sp.	0/3 (0%)	1/2 (50%)	2/15 (13.3%)	NA	NA	0/1 (0%)	NA	NA
Phytophagous	<i>Platyrrhinus</i> sp.	NA	0/1 (0%)	0/1 (0%)	NA	NA	1/2 (50%)	NA	NA
	<i>Dermanura glaucus</i>	NA	0/1 (0%)	NA	NA	NA	NA	NA	NA
	<i>Rhinophylla pumilio</i>	NA	0/2 (0%)	NA	NA	NA	NA	NA	NA
	<i>Uroderma bilobatum</i>	NA	0/1 (0%)	NA	NA	NA	0/1 (0%)	NA	NA
	<i>Vampyriscus bidens</i>	NA	2/3 (66.7%)	NA	NA	NA	NA	NA	NA
	<i>Vampyressa thuyone</i>	NA	NA	NA	NA	NA	1/2 (50%)	NA	NA
	<i>Monophyllus redmani</i>	NA	NA	NA	NA	7/9 (78%)	NA	NA	NA
	<i>Brachyphylla cavemmarum</i>	NA	NA	NA	NA	1/1 (100%)	NA	18/25 (72%)	NA
	<i>Lonchophylla robusta</i>	NA	NA	NA	NA	NA	0/3 (0%)	NA	NA
	<i>Hylonycteris underwoodi</i>	NA	NA	NA	NA	NA	0/2 (0%)	NA	NA
	<i>Enchisthenes hartii</i>	NA	NA	NA	NA	NA	0/1 (0%)	NA	NA
	<i>Ardops nichollsii</i>	NA	NA	NA	NA	NA	NA	5/25 (20%)	NA
	<i>Erophylla sezekorni</i>	NA	NA	NA	NA	0/14 (0%)	NA	NA	NA

The common vampire bat feeds primarily on livestock in Mexico, and is potentially exposed to blood-borne pathogens due to its dietary niche. However, no *Bartonella* strains currently described from *D. rotundus* hosts overlap with any *Bartonella* species known to infect ruminants. At this point, there is no evidence of transmission or spillover of *Bartonella* between vampire bats and their prey, though this requires further research. Continued climate change is also potentially expanding the range of *D. rotundus* to higher altitudes and more northern regions, which may soon overlap with southern Texas.³¹ The common vampire bat is already a major reservoir of rabies in Latin America and a better understanding of the pathogen diversity harbored by *D. rotundus* may better aid in future disease management strategies.

Given that *Bartonella* variants have also been detected from ectoparasitic bat flies, it is possible that these obligate ectoparasites vector the bacteria within and between bat populations.^{32,33} After adjusting for age and diet, statistical analysis revealed a positive association with the number of ectoparasites present on a bat host and odds of *Bartonella* infection. These findings support the phylogenetic studies that have identified shared bartonellae in both bats and their ectoparasites.^{14,32–39}

The bat-associated *Bartonella* strains isolated in Mexico also clustered with those previously described in bats and ectoparasites from other countries in Latin America. However, this is the first identification of *Bartonella* species in *B. plicata*, recognizing another bat genus to be infected with bartonellae. In Guatemala, a total of 39 (33.1%) *Bartonella* isolates were obtained from 118 bat samples, with approximately half (48.4%) of the *D. rotundus* tested *Bartonella* positive.²⁶ In Peru, 24.1% of 112 bats sampled were *Bartonella* positive, with common vampire bats having a prevalence of 55.6%.²² This study in Mexico revealed similar findings, with a total *Bartonella* prevalence of 22.7% across all bat species and 38% in *D. rotundus* (Table 6). In French Guiana, eight (13.6%) of 59 bats were PCR positive for *Bartonella* spp.,³⁷ 33.3% (21/63) of the bats sampled in southern Costa Rica were also positive for *Bartonella* spp. DNA,²⁴ and in Saint Kitts island.³⁹ In Puerto Rico, *Bartonella* isolates were obtained from blood specimens of 9/51 (18%) individuals from three species (*A. jamaicensis*, *Brachyphylla cavernarum*, and *Monophyllus redmani*).²⁵ Across studies, *Bartonella* prevalence was the highest in hematophagous bats (42%, 66/158), followed by phytophagous (22.9%, 84/367) and insectivorous bats (19.3%, 42/217).

In general, the numbers of *Bartonella* sequences described from diverse bat hosts and related ectoparasites around the world have set the foundation for a better epidemiological understanding of pathogen evolution, maintenance, and transmission in these systems. Comparative host–pathogen coevolutionary analyses of *Bartonella* in bat hosts from the existing literature have already provided evidence for overall evolutionary congruence between both Old World and New World bat hosts and their respective bartonellae.^{40,41} Generally, as vector-transmitted pathogens, *Bartonella* is influenced by an additional evolutionary mechanism while adapting to both an arthropod organism and a mammalian host. This can exert selective pressures that may drive speciation of the bacteria. Further elucidating these factors in bat-associated *Bartonella* can provide key information to better understand why certain bats are infected with specific *Bartonella* variants and how they are transmitted.

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