

Molecular Detection of *Candidatus Bartonella mayotimonensis* in North American Bats

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Abstract

Candidatus Bartonella mayotimonensis was detected in 2010 from an aortic valve sample of a patient with endocarditis from Iowa, the United States of America. The environmental source of the potentially new endocarditis-causing *Bartonella* remained elusive. We set out to study the prevalence and diversity of bat-associated *Bartonella* in North America. During 2015, mist nets and harp traps were used to capture 92 bats belonging to two species: little brown myotis (*Myotis lucifugus* Le Conte 1831, $n=73$) and the gray myotis (*M. grisescens* A.H. Howell 1909, $n=19$) in Kentucky, Michigan, Pennsylvania, and Tennessee. DNA preparations of peripheral blood samples from bats were subjected to a three-marker (*gltA*, *rpoB*, and intergenic spacer region [ISR]) multilocus sequence analysis. Sequence-verified *gltA*-positive PCR amplicons were obtained from nine samples. Three sequences were 99.7–100% identical with the *gltA* sequence of the Iowa endocarditis patient strain. Analysis of *rpoB* and ISR sequences demonstrated that one little brown myotis sample from the Upper Peninsula of Michigan contained *Bartonella* DNA, with 100% sequence identity with the Iowa endocarditis patient strain DNA. It appears possible that bats are a reservoir of *Candidatus Bartonella mayotimonensis* in North America.

Keywords: *Bartonella*, bats, Chiroptera, endocarditis

Introduction

BARTONELLA IS A genus of gram-negative, facultative intracellular, and hemotrophic bacteria, the species of which infect a range of mammals worldwide (Pulliainen and Dehio 2012). Some species of *Bartonella* are potent human pathogens (e.g., *B. quintana* and *B. henselae*), which are frequent in cases of inflammation of the inner surface of the heart (endocarditis) (Edouard et al. 2015), and new pathogenic species are emerging. For example, a new type of *Bartonella* (*Candidatus Bartonella mayotimonensis*) was detected in 2010 from an aortic valve sample of an endocarditis patient from Iowa, the United States of America (Lin et al. 2010).

[Veikkolainen et al. \(2014\)](#) recently detected and isolated *Bartonella* strains from peripheral blood samples of Dau-

benton's myotis (*Myotis daubentonii* Kuhl 1817) in Southwestern Finland. Multilocus sequence analysis (MLSA) demonstrated that *rpoB* and *ftsZ* markers of the isolated strains had up to 96–97% sequence identity with the Iowa *Candidatus B. mayotimonensis* patient strain sequences ([Veikkolainen et al. 2014](#)). However, one frequently used *Bartonella* MLSA marker, the *gltA* ([Norman et al. 1995](#)), displayed low values of sequence identity (91.4–92.9%) ([Veikkolainen et al. 2014](#)). This casts doubt on the possibility that the *Bartonella* isolates from Finnish bats were strains of *Candidatus B. mayotimonensis*.

Here, we set out to study the prevalence and diversity of bat-associated *Bartonella* in North America, where *Candidatus B. mayotimonensis* was first detected from the endocarditis patient sample.

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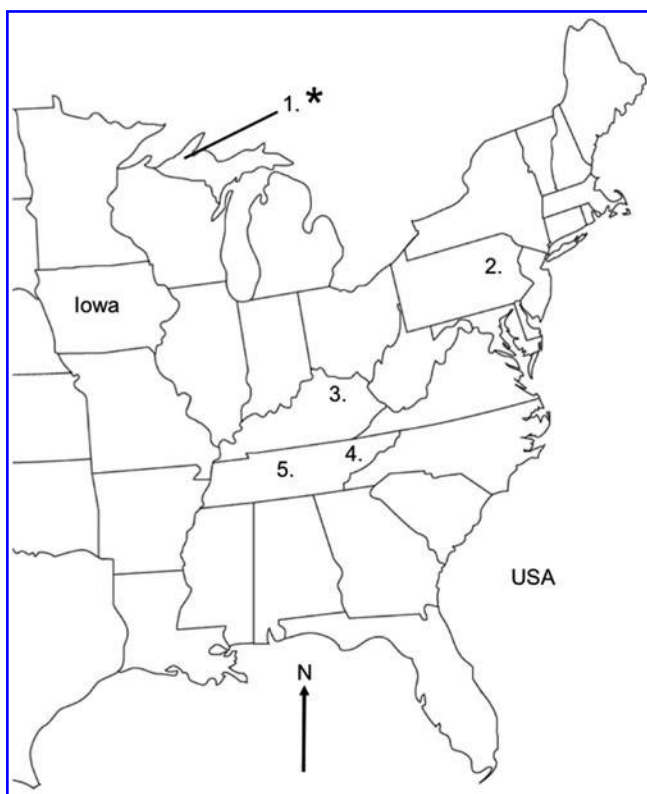


FIG. 1. Study locations in the eastern United States of America. Bats were captured in five locations. Asterisk refers to the location where strains of *Candidatus Bartonella mayotimonensis* were detected. The initial endocarditis patient with *Candidatus B. mayotimonensis* infection was diagnosed in the state of Iowa (Lin et al. 2010). Numbers after locations represent *Bartonella*-positive bats/total number of bats caught: (1) Greenland, Upper Peninsula, Michigan (7/43); (2) Allentown, Pennsylvania (0/24); (3) Frankfort, Kentucky (0/6); (4) Warren County, Tennessee (1/7); (5) Montgomery County, Tennessee (1/12).

Materials and Methods

During 2015, mist nets and harp traps were used to capture bats at hibernacula or summer roosts in Kentucky, Michigan, Pennsylvania, and Tennessee. Ninety-two bats belonging to two species, the little brown myotis (*Myotis lucifugus* Le Conte 1831, $n=73$) and gray myotis (*M. grisescens* A.H. Howell 1909, $n=19$), were included in this study (Fig. 1 and Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/vbz). The uropatagium was cleaned with an alcohol wipe; subsequently, the interfemoral vein was lanced with a 27-G needle, and blood was drawn into a 75- μ L heparinized capillary tube. Tubes were centrifuged (5 min at 5000 rpm), and packed cells were diluted in 300 μ L of phosphate-buffered saline (PBS), flash frozen in liquid nitrogen, and stored at -80°C .

All methods were approved by the Institutional Animal Care and Use Committee at Bucknell University (protocol DMR-016). The bats were collected under Scientific Collector's Permits from Michigan (SC1475) and Kentucky (SC1411147), Pennsylvania Game Commission Special Use Permit (permit #33085), Tennessee Wildlife Resource Agency Scientific Collection Permit (#3742), and USFWS permit # TE35313B.

DNA was isolated from 100 μ L of the PBS-diluted packed cell sample by using a QIAamp DNA mini kit (Qiagen; 51304) for a final elution of 60 μ L. The rest of the packed cell samples (200 μ L) were used for bat ecophysiology studies that are neither related to nor reported in this study. Therefore, we did not make attempts to cultivate *Bartonella* strains.

Subsequently, 5 μ L of the isolated DNA solution was subjected to a master mix-based PCR protocol that was specific for the citrate synthase gene (*gltA*) (Norman et al. 1995), for the RNA polymerase B-subunit gene (*rpoB*), and for the 16S-23S rRNA intergenic spacer region (ISR) (García-Esteban et al. 2008) with DyNAzyme II DNA polymerase (Thermo Fischer Scientific; F501S) (Table 1). Primers specific for *rpoB* (Table 1) were custom-designed to match the sequence of the strain from the patient from Iowa (Lin et al. 2010), and they are applicable, for example, as a nested oligo pair when the

TABLE 1. OLIGONUCLEOTIDE PRIMERS AND POLYMERASE CHAIN REACTION PROGRAM USED IN THE *BARTONELLA* MULTILOCUS SEQUENCE ANALYSIS

Oligo	Target genetic marker, oligo orientation	Sequence 5' \rightarrow 3'	Reference
Bart/16-23F	16S-23S rRNA intergenic spacer region (ISR), forward	TTGATAAGCGTGAGGTCGGAGG	García-Esteban et al. (2008)
Bart/16-23R	16S-23S rRNA intergenic spacer region (ISR), reverse	CAAAGCAGGTGCTCTCCCAG	García-Esteban et al. (2008)
BhCS.781p	GltA gene, forward	GGGGACCAGCTCATGGTGG	Norman et al. (1995)
BhCS.1137n	GltA gene, reverse	AATGCAAAAAGAACAGTAAACA	Norman et al. (1995)
prAPV-132	RpoB gene, forward	GATGTACATCCTACGCATTATGG	This study
prAPV-133	RpoB gene, reverse	AATGGTGCTTCAGCACGTACAAG	This study
	1. Initial denaturation	95°C, 1 min	
	2. Denaturation	95°C, 30 s	
	3. Annealing	55°C, 40 s	
	4. Extension (number of cycles 2-4)	72°C, 1 min (40 cycles)	
	5. Final extension	72°C, 10 min	
	6. Stay	4°C, forever	

TABLE 2. THE PERCENT OF HOMOLOGY BETWEEN *gltA*, *rpoB* AND INTERGENIC SPACER REGION SEQUENCES OBTAINED FROM THREE LITTLE BROWN MYOTIS SAMPLES FROM THE UPPER PENINSULA OF MICHIGAN AND THE IOWA *CANDIDATUS* *BARTONELLA* MAYOTIMONENSIS PATIENT STRAIN

	rpoB	gltA	ISR
Bat-DMR02051	99.7% (337/338 bp)	100% (406/406 bp)	95.4% (270/278 bp)
Bat-DMR02005	100% (338/338 bp)	100% (406/406 bp)	100% (283/283 bp)
Bat-DMR02028	100% (338/338 bp)	98.8% (401/406 bp)	92.9% (263/283 bp)

ISR, intergenic spacer region.

frequently used *rpoB*-specific 1400F/2300R oligo pair (Re-[nesto et al. 2001](#)) has been utilized in primary PCR. Distilled water and 10 ng of chromosomal DNA of *B. quintana* strain Toulouse were used as the negative and positive controls, respectively.

Results

Sequence-verified *gltA*-positive PCR amplicons (GenBank KX807171–KX807179) were obtained from nine samples (Supplementary Table S1). Searches using BLAST (August 2016) with the *gltA* sequences demonstrate that samples from three little brown myotis from the Upper Peninsula of Michigan contained DNA of *Bartonella* that was most similar (97.5%) to DNA of *Bartonella* previously detected in a bat fly, *Trichobius corynorhini*, in the United States of America (GenBank JX41623; unpublished). One sample from the little brown myotis from the Upper Peninsula of Michigan and one from the gray myotis from Tennessee contained DNA of *Bartonella* that was most similar (98.0%) to DNA of *Bartonella* previously detected in Costa Rican bats (GenBank KJ816689; unpublished). One sample from a gray myotis from Tennessee contained DNA of *Bartonella* that was only distantly related to any entry in GenBank. This sample contained DNA that was closest (91%) to the DNA of *Bartonella* DNA that was previously detected in samples from Costa Rican bats (GenBank KJ816665; unpublished).

Remarkably, blood from three little brown myotis from the Upper Peninsula of Michigan contained DNA of *Bartonella*, with up to a 100% sequence identity with the strain of *Candidatus B. mayotimonensis* from Iowa ([Lin et al. 2010](#)) (Table 2). We further subjected these three samples to master mix-based PCR protocols that were specific for the *rpoB* and ISR ([García-Esteban et al. 2008](#)) (Table 1). Analysis of *rpoB* and ISR sequences (GenBank KX807182–KX807187) demonstrated that one sample from a little brown myotis from the Upper Peninsula of Michigan contained DNA of *Bartonella*, with up to a 100% sequence identity with the strain of *Candidatus B. mayotimonensis* from the patient in Iowa ([Lin et al. 2010](#)) (Table 2).

Discussion

Based on our results, we suggest that bats are a reservoir of *Candidatus Bartonella mayotimonensis* in North America. We found out that the *gltA*, *rpoB*, and ISR MLSA marker sequences of some *Bartonella* strains associated with North American bats are 100% identical with the *Bartonella* detected in the patient with endocarditis from Iowa, the United States of America ([Lin et al. 2010](#)). This patient lived on a farm, and it is possible that this person could have come into

contact with bats roosting in one or several farm buildings. The little brown myotis often roosts in attics or barns during summer, and the size of their maternity colonies can reach hundreds of individuals. During weaning, pups often stray inside houses and may come into contact with humans.

One possible transmission mechanism of hemotrophic *Bartonella* from bats to humans may be bat ectoparasites or their feces. We have detected *Bartonella gltA* sequences (GenBank KX807180–KX807181) in Canada from bat-associated *Cimex adjunctus* (unpublished). These sequences were most similar (98.2% and 97.3%) to DNA of *Bartonella* previously detected in Costa Rican bats (GenBank KJ816689; unpublished) and *Bartonella* DNA previously detected in a bat fly *Trichobius corynorhini* in the United States of America (GenBank JX416236; unpublished). A previous report describes the detection of *Bartonella* sp. DNA in one bat bug, *C. adjunctus*, from South Carolina, the United States of America ([Reeves et al. 2005](#)). The presence of human blood in an engorged bat tick, *Carios kelleyi*, from Iowa has also been reported ([Gill et al. 2004](#)), indicating that this bat ectoparasite may occasionally feed on human blood. Remarkably, *Bartonella* sp. DNA has been detected in *C. kelleyi* in Iowa, the United States of America ([Loftis et al. 2005](#)).

More studies on the presence of viable *Bartonella* in bat-associated ectoparasites are needed to critically evaluate whether bat ectoparasites or their feces are involved in the transmission of hemotrophic *Bartonella* to humans. Moreover, the cultivation of North American isolates of *Candidatus Bartonella mayotimonensis* from bat peripheral blood samples should be attempted, for example, similar to our previous study conducted in Finland ([Veikkolainen et al. 2014](#)). The isolated strains would allow detailed multilocus sequence analytics, genome sequencing, as well as the development of serological tests for the assessment of the clinical importance of *Candidatus Bartonella mayotimonensis*.

Following the pioneering work by the Birtles laboratory in the United Kingdom ([Concannon et al. 2005](#)), our group in Finland ([Veikkolainen et al. 2014](#), [Lilley et al. 2015](#)), among others, has shown that a diversity of *Bartonella* genotypes are associated with bats and their ectoparasites on a global scale, including Kenya ([Kosoy et al. 2010](#)), Guatemala ([Bai et al. 2011](#)), Peru ([Bai et al. 2012](#)), and Taiwan ([Lin et al. 2012](#)). In fact, the non-*Candidatus B. mayotimonensis gltA* sequences detected in this study have less than 95% sequence identity with any of the *Bartonella* type strain *gltA* sequences. This indicates that bats in North America may host a plethora of new species of *Bartonella*. Given the possible link between bat-associated *Bartonella* and endocarditis, more studies similar to [Mannerings et al. \(2016\)](#) are needed to assess the zoonotic potential of bat-associated *Bartonella*.

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Author Disclosure Statement

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