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BRIEF REPORT

Bartonella spp. in different species of bats from Misiones (Argentina)

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Abstract The aim of this study was to detect vector-borne pathogens (Anaplasmataceae family, *Rickettsia* genus, and *Bartonella* genus) in bats from Misiones (Argentina). Thirty-three specimens were captured over 8 days using mist nets. Twenty (60.6%) blood samples were positive (11/13 *Artibeus lituratus*, 4/10 *Desmodus rotundus*, 4/8 *Carollia perspicillata*, and 1/2 *Myotis nigricans*) by PCR for the *gltA* gene fragment of *Bartonella*. All samples were negative by PCR for the *Anaplasmataceae* family and *Rickettsia* genus.

The phylogenetic analysis showed seven *Bartonella* genotypes. The three genotypes obtained from *A. lituratus*, 2 from *C. perspicillata*, and 1 from *D. rotundus* were related to *Bartonella* spp. from New World bats, while the sequence obtained from *M. nigricans* was related to Old World bats.

We identified a considerable diversity of *Bartonella* genotypes in a small number of bats, thus further research is required to better understand the complex bat–pathogen interaction.
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PALABRAS CLAVE
Bartonella;
Murciélagos;
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Argentina

Circulación de *Bartonella* spp. en diferentes especies de murciélagos en la provincia de Misiones (Argentina)

Resumen El objetivo de este estudio fue detectar patógenos transmitidos por vectores (familia *Anaplasmataceae*, género *Rickettsia* y género *Bartonella*) en murciélagos de Misiones (Argentina). Se capturaron 33 ejemplares con redes de niebla. Veinte (60,6%) muestras de sangre fueron positivas a una reacción en cadena de la polimerasa (PCR) para un fragmento del gen *gltA* de *Bartonella* (11/13 en muestras tomadas de ejemplares de la especie de murciélago *Artibeus lituratus*, 4/10 en *Desmodus rotundus*, 4/8 en *Carollia perspicillata* y 1/2 en *Myotis nigricans*). Todas las muestras resultaron negativas para la familia *Anaplasmataceae* y el género *Rickettsia*.

El análisis filogenético demostró siete genotipos de *Bartonella*. Los tres genotipos obtenidos de *A. lituratus*, 2 de *C. perspicillata* y 1 de *D. rotundus* estuvieron relacionados con *Bartonella* spp. de murciélagos del Nuevo Mundo, mientras que la secuencia obtenida de *M. nigricans* se relacionó con secuencias del Viejo Mundo. Es necesario estudiar un mayor número de ejemplares para comprender mejor la compleja interacción entre murciélagos, vectores y patógenos.
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Bats are the second most diverse order of mammals globally. There are more than 1400 species distributed throughout the world except in the poles[2]. Bats are susceptible to different microorganisms, including viruses, bacteria, fungi and parasites, as well as to pathogens of importance in human and animal health[15].

The province of Misiones (Argentina) is part of the Paranaense jungle and Campos and Malezales ecoregions due to the characteristics of its climate, soil and vegetation[14]. Misiones has the greatest diversity of bat species in the country, belonging to the families *Noctilionidae*, *Phyllostomidae*, *Vespertilionidae* and *Molossidae*[2,3,13,17], with the presence of 42 species in the province out of a total of 70 species in Argentina. These families include carnivorous, piscivorous, frugivorous, insectivorous, nectarivorous and hematophagous bats[2].

Bats have been implicated as reservoir hosts for diverse microorganisms, some of which are pathogenic to humans. Vector-borne bacteria (*Bartonella*, *Rickettsia*, *Borrelia* and *Neorickettsia risticii*) have been detected in blood and organ tissues of bats around the world[15]. Host-switching and variations in host-specificity of ectoparasites are important factors in these pathogens, with a potential risk of occurrence of epidemiological cycles of transmission between bats, humans and domestic animals[15].

The only reports of vector-borne bacterium in bats from Argentina was in the specie *Tadarida brasiliensis* (*Molossidae* family) from Buenos Aires City[5]; therefore, the aim of the present study was to detect vector-borne pathogens (*Anaplasmataceae* family, genus *Rickettsia*, and genus *Bartonella*) in different species of bats from Misiones (Argentina).

Sampling was performed during 8 days throughout the months of November 2015 and April 2016 in AICOM A-A003 (Spanish acronym for Area of Importance for Bat Conservation) known as Osununú/Teyú Cuaré, which involves the Teyú Cuaré Provincial Park and Osununú Private Reserve, which is located near San Ignacio village, Misiones Province, Argentina (27°17′04.53″S–55°35′19.44″). Bat capture was conducted using six mist nets with a length of 6–12 m and a height of 4 m, which remained open from 6 pm to 4 am on dark moon nights[20]. Thirty-three bats were captured and identified according to Barquez and Díaz, 2009[1]: *Artibeus lituratus* (13), *Desmodus rotundus* (10), *Carollia perspicillata* (8) and *Myotis nigricans* (2). The four species of bats studied are widely distributed throughout South America; however, *M. nigricans* and *A. lituratus* have not been reported in Chile and Uruguay, respectively[6]. *Artibeus lituratus* and *C. perspicillata* are frugivorous, *M. nigricans* are insectivorous and *D. rotundus* is one of the three South American species that feeds exclusively on blood[2].

Blood samples were taken from the brachial vein, not exceeding 1% of the total body weight, using non-heparinized 1 ml tuberculin syringes with 27GX1/2″ needles[7]. DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany), following the manufacturer's instructions. For each sample, three individual polymerase chain reactions (PCR) were performed for the screening of the *Anaplasmataceae* family, genus *Rickettsia*, and genus *Bartonella*[8,9,18]. *Anaplasma centrale*, *Rickettsia conorii*, and *Bartonella clarridgeiae* were used as positive control, respectively. Nuclease-free water was used as negative control.

Amplicons were randomly selected (representing a minimum of 50% of the findings per bat species) to be sequenced. Purification from agarose was performed using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Irvine, USA) and sequenced with a 3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, USA). The sequences were edited using BioEdit Sequence Alignment Editor and aligned with the Clustal W program, and compared with sequences deposited in GenBank. A phylogenetic analysis was performed using the maximum-likelihood (ML)

Table 1 Bats testing positive for the genus *Bartonella*.

Bat species	Family	Number of specimens	Positives for *Bartonella*		Sequences obtained	Identity with each other
			n	%		%
Artibeus lituratus	Phyllostomidae	13	11	84.6	6	81.5–100
Desmodus rotundus	Phyllostomidae	10	4	40.0	2	100
Carollia perspicillata	Phyllostomidae	8	4	50.0	2	85.3
Myotis nigricans	Vespertilionidae	2	1	50.0	1	–
Total		33	20	60.6	11	81.5–100

method and the best-fitting substitution model was determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 6.0. Support for the topologies was tested by bootstrapping over 1000 replications and gaps were excluded from the comparisons.

All samples tested negative by the PCRs for the 16S rRNA fragment of the *Anaplasmataceae* family and 23S-5S intergenic spacer fragment of the *Rickettsia* genus. Previous studies have detected *N. risticii* (*Anaplasmataceae* family) in bats of the *Myotis* genus from USA and *T. brasiliensis* in bats from Buenos Aires City (Argentina)[5]. Moreover, only two studies reported *Rickettsia* spp. in bats by PCR, including one in *T. brasiliensis* from Buenos Aires City (Argentina)[5].

Twenty (60.6%) samples tested positive by PCR for the *gltA* gene fragment of *Bartonella* and 11 (55.0%) were sequenced (Table 1). The sequences obtained exhibited 81.5–100% identity among them (Table 1 and Fig. 1). The ML phylogenetic tree generated with *gltA* sequences evidenced seven different potential genotypes of *Bartonella* (3 from *A. lituratus*, 2 from *C. perspicillata*, 1 from *D. rotundus* and 1 from *M. nigricans*), which are associated with different groups related to previous findings in bats (Fig. 1).

To our knowledge, this is the first finding of *Bartonella* spp. in the above species of bats from Argentina and the first detection in *M. nigricans*. Based on the *gltA* molecular marker, we found a high diversity of *Bartonella* in the bat species studied, which is consistent with previous reports. *Bartonella* spp. found in the *Phyllostomidae* family were related to previous findings from the New World family (South and Central America), while the *Bartonella* sp. from *Vespertilionidae* (*M. nigricans*) was grouped with sequences from that family from the Old World.

There are numerous reports of *Bartonella* spp. in different species of bats from America[4,12,16,19]. It should be noted that these studies showed different samples (blood, organs) and methodologies (PCR, culture, molecular characterization), as well as a variable number of samples studied[4,12,16,19].

Bartonella spp. were previously reported in *A. lituratus* from Costa Rica and Brazil[4,12,19]. Two sequences obtained in our study from *A. lituratus* (M122 and M129, GenBank accession numbers MZ005985 and MZ019482, respectively) showed 100% identity among them and with *Bartonella* spp. associated to *A. lituratus* from Costa Rica (MH234317, MH234324 and MH234326). Two other sequences from the same bat species (M117 and M118, GenBank accession numbers MZ005987 and MZ005986, respectively) exhibited 100% identity among them and 99.6% with *Bartonella* spp. of *Artibeus jamaicensis* from Guatemala (MN529479) and the bat fly from Mexico (MF988085). The remaining two sequences obtained from *A. lituratus* (M124 and M126, GenBank accession numbers MZ005984 and MZ005983, respectively) showed 95.4% identity with *Bartonella* sp. from *Sturnira lilium* (Mexico) (KY629850) and 100% identity with *Bartonella* sp. from *A. lituratus* (Costa Rica) (MH234318), respectively.

In *C. perspicillata*, *Bartonella* spp. were detected in Guatemala, Costa Rica, Peru and Brazil[4,12,19]. In our analysis, one sequence obtained (M139, GenBank accession number MZ019484) is closely related to a group of sequences from *Carollia* spp. and *Artibeus* spp. from Peru, Brazil, Costa Rica and Guatemala, and the other (M116, GenBank accession number MZ005990) is related to a group of sequences from different bat species (*D. rotundus* and *A. lituratus*) and bat flies from Peru, Brazil, Costa Rica and Belize (Fig. 1).

Bartonella spp. associated to *D. rotundus* were found in Brazil, Peru, Belize, Mexico, Costa Rica and Guatemala[4,12,19]. The sequences found in *D. rotundus* (M110 and M112, GenBank accession numbers MZ005988 and MZ005989, respectively) showed 100% identity among them, and with *Bartonella* sp. of *D. rotundus* of Peru (MG799415).

The only sequence obtained from *M. nigricans* (M134, GenBank accession number MZ019483) showed 96.5% identity with the *Bartonella* spp. found in *Nycteribia kolenatii* bat fly from Romania (MK140280) and Belgium (MK140265), and *Myotis blythii* from Georgia (MK140355), among others. In America, *Bartonella* spp. were detected in *Myotis chiloensis* from Chile[16], *Myotis* sp. from Peru[19], *Myotis keaysi* from Costa Rica[19] and *Myotis lucifugus* and *Myotis grisescens* from USA[10]. All these findings are not phylogenetically related to the *Bartonella* sp. found in *M. nigricans* in our study. Finally, the previous report of *Bartonella* sp. in bats (species *T. brasiliensis*, family *Molossidae*) from Argentina[5] is not phylogenetically related to the findings of the present study.

With regard to their significance in human and animal health, the species *Bartonella* found in our study are of unknown pathogenicity. However, other authors have detected a *Bartonella* sp. in bats that has been associated with endocarditis in humans[10].

Different clades of *Bartonella* found in bats are related to families, superfamilies and suborders of bats, suggesting co-divergence between bats and *Bartonella*[11,15]. In our study, a significant diversity of *Bartonella* species was identified in a small number of bats sampled, revealing a complex host-pathogen interaction. Thus, further research is required to better understand the circulation of vector-borne pathogens

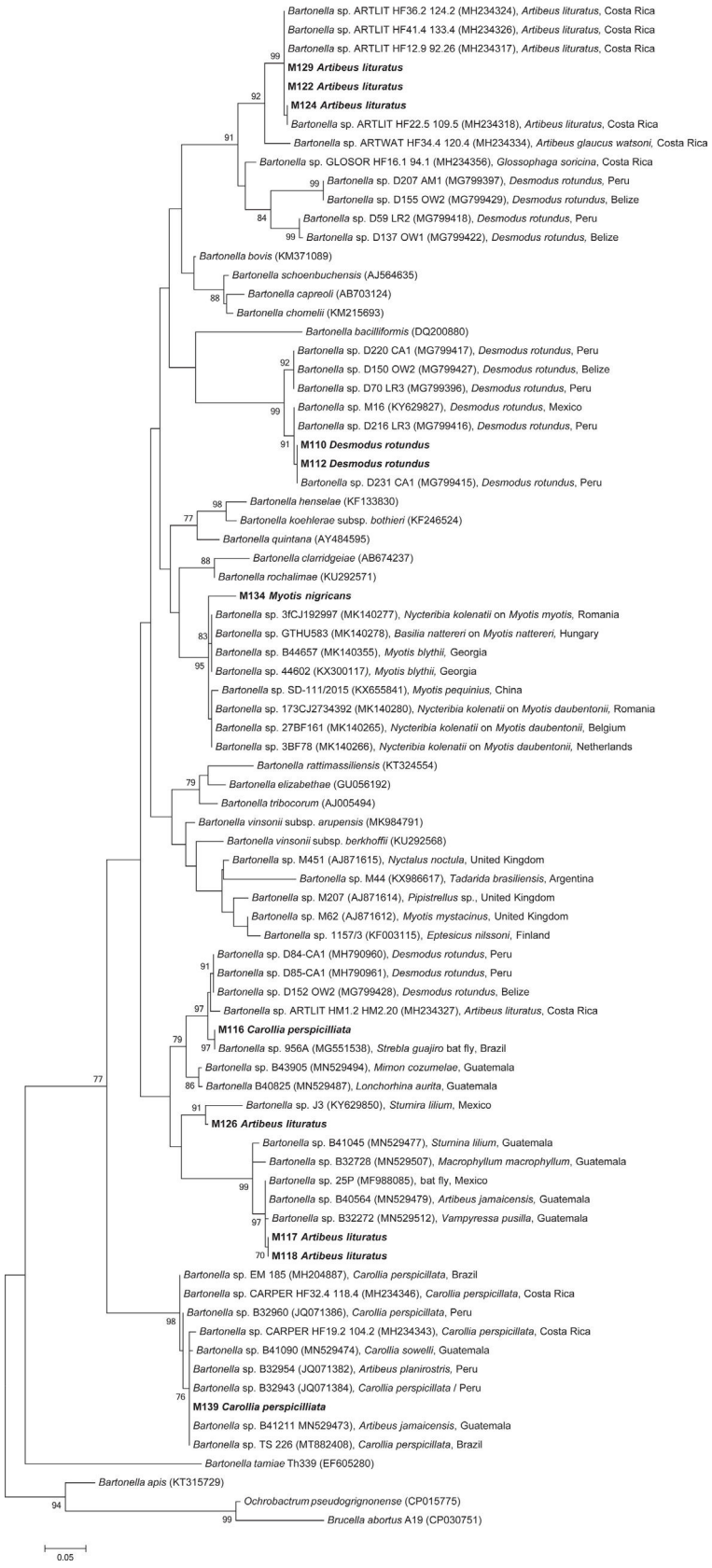

Figure 1 Maximum-likelihood tree constructed using the Tamura 3-parameter model (+G) from partial sequences of *Bartonella gltA*. Numbers represent bootstrap support generated from 1000 replications (only bootstrap support >70 is shown). GenBank accession numbers are in brackets. Additional information (host and country) is provided.

in bats and their ectoparasites. Sampling at different times of the year should be conducted to gain deeper insight into the host-pathogen relationship, as well as into the occurrence of spillover of *Bartonella* species into other wild and domestic animals, and the potential risk for human populations.

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Authors' contributions

A.P. and A.D.R. fieldwork; M.N.D.S. and G.L.C. lab work and phylogenetic analysis; A.P., M.N.D.S. and G.L.C. writing – original draft preparation; I.L.R, M.N.D.S. and G.L.C. writing – review and editing; F.J.B. and F.E.G.D. resources administration and supervision. All authors reviewed the manuscript.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The DNA sequences generated and analyzed during the current study are available in the GenBank repository [https://www.ncbi.nlm.nih.gov/nucleotide/].

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