



## Correspondence

### **Molecular identification of zoonotic *Rickettsia* species closely related to *R. typhi*, *R. felis*, & *R. rickettsii* in bats from Mexico**

Rickettsioses are a zoonoses characterized by fever and exanthematic syndrome and has become an important public health problem in Mexico<sup>1</sup>. Several outbreaks in humans have been reported in the northern and southeastern States with a fatality as high as 30-80 per cent, when the diagnosis is delayed<sup>1,2</sup>. The disease is caused by a species of the *Rickettsia* genus transmitted mainly by ticks, that get in close contact with patients due to recreational or economic activities, or by close contacts with domestic and wild animals such as dogs, cats, opossums, small rodents and bats<sup>1</sup>. Nevertheless, the ecological dynamics of the spread of *rickettsial* infections are not fully understood. Flight capabilities, among other characteristics, suggest that bats could serve as vector dispersers and potential pathogen reservoirs as it is known from birds<sup>3</sup>. There are reports of ticks parasitizing bats (genera *Argas* and *Ornithodoros*) that are infected with *R. bellii*, *R. africae*, *R. felis*, and *R. lusitaniae*, among others<sup>3-5</sup>. There are few reports that describe a *Rickettsia*-associated bacteraemia in bats, but little is known about its implications in zoonotic transmission<sup>3-9</sup>. To date, only one report from the Yucatan peninsula has been published about ticks from the *Ornithodoros* genus collected in a bat cave that were infected with *R. lusitaniae*<sup>7</sup>. The aim of this study was to investigate the presence of *Rickettsia* in bats from a suburban area of Mexico using molecular methods.

The permission to capture bats was obtained from the Bioethics Committee of the Faculty of Veterinary Medicine (FMVZ), Campus of Biological & Agricultural Sciences (CCBA) of the Autonomous University of Yucatan (UADY) and the Ministry of Environment and Natural Resources (SEMARNAT) from Mexico. Bats were captured in X'matkuil, Yucatan (20.865927-89.623794) during September 2018 with three mist nets (12×2.6 m). This area is part of the south

suburban area of the city of Merida, Yucatan, in which several cases of rickettsiosis have been documented<sup>1,10</sup>. Somatic measurements, age (juvenile or adult), species, and sex were recorded<sup>11</sup>. All bats were euthanized to collect the spleen, which was used for DNA extraction using the Wizard Genomic A1125 kit (Promega®, USA) according to the manufacturer's standard instructions, with 20 mg of tissue. PCR analyses were performed with 200 ng of extracted DNA using primers and conditions as reported previously<sup>10</sup>.

A nested PCR was performed targeting *OmpB* using the primers rOmpB-OF and rOmpB-OR in the first round (for *Rickettsia* genus); followed by a second-round using a mixture of the primers rOmpBSFG IF, rOmpB TG IF and rOmpB SFG/TG IR (for group correspondence of the *Rickettsia* amplified in the first round)<sup>12</sup>. For PCR detection of the 17 kDa lipoprotein antigen (*htrA*) gene, primers FwRr1175F and Rr2608R were used<sup>13</sup>. Positive PCR products of both genes were purified with columns (Qiagen-PWS288, USA), sequenced by the Sanger method (DIMYGeN, Mexico), and sequences were compared to entries in the GenBank database using the software BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and MEGA (<https://megasoftware.net/>).

A total of 22 Mexican fruit bats (*Artibeus jamaicensis*) were captured and subjected to necropsy. No ticks, mites or fleas were found during the external inspection. None of the bats showed signs of a clinical infection or relevant abnormalities in weight, size or physical condition. Nine bats were PCR-positive for the *OmpB* and *htrA* genes (40.9%) (Table). Sequence analyses showed that eight bats were infected by *Rickettsia* related to the SFG. In four bats, the *OmpB* and *htrA* gene sequences were closely related to those of *R. rickettsii* (299 bp, 93% coverage, 100% identity for *OmpB*; and 434 bp, 100% coverage, 100% identity for *htrA*), while sequences of *OmpB*

**Table.** Results of sequence analyses of the nested PCR product of the spleen DNA extract from the captured bats

BAT Id	Sex	Age	<i>OmpB</i> (percentage sequence identity)	GenBank accession number	<i>htrA</i> (percentage sequence identity)	GenBank accession number	GenBank accession numbers for closest sequences ( <i>OmpB</i> / <i>htrA</i> )	Closest homology
M1	Female	Adult	100	MT462103	100	MT462099	X16353.1 AY281069.1	<i>R. rickettsii</i>
M2	Male	Juvenile	96	MT462108	98	MT462107	HQ236390.1 AAU03599.1	<i>R. typhi</i>
M5	Female	Adult	100	MT463320	99	MT463319	GQ385243.1 APO14768.1	<i>R. felis</i>
M9	Male	Juvenile	100	MT462105	100	MT4620100	X16353.1 AY281069.1	<i>R. rickettsii</i>
M15	Male	Juvenile	100	MT462104	100	MT4620101	X16353.1 AY281069.1	<i>R. rickettsii</i>
M16	Male	Adult	100	MT463321	99	MT463316	GQ385243.1 APO14768.1	<i>R. felis</i>
M17	Female	Adult	100	MT463317	99	MT463322	GQ385243.1 APO14768.1	<i>R. felis</i>
M18	Male	Juvenile	100	MT462106	100	MT4620102	X16353.1 AY281069.1	<i>R. rickettsii</i>
M19	Male	Juvenile	100	MT463318	99	MT463323	GQ385243.1 APO14768.1	<i>R. felis</i>

*R. rickettsia*, *Rickettsia rickettsia*

and *htrA* from another four bats were closely related to *R. felis* (220 bp, 100% coverage, 100% identity for *OmpB*; 341 bp, 100% coverage, 99% identity for *htrA*). In addition, sequences of *OmpB* and *htrA* from a single bat were closely related to *R. typhi* (367 bp, 99% coverage, 96% identity for *OmpB*; 334 bp, 100% coverage, 98% identity for *htrA*). No evident differences were observed between the sex or age of the infected bats (*P* data >0.05 therefore is not shown) (Table).

Bats are members of one of the biggest and widely dispersed groups of animals in the world. Their flight capabilities allow them to cover long distances and their natural behaviour to cluster in roosts with close contact among individuals, could favour the spread of ectoparasites and its pathogens<sup>4</sup>. Several studies investigated the role of bats in the transmission of infectious agents, including vector-borne bacteria such as *Bartonella*, *Anaplasma*, and *Borrelia*<sup>4,6</sup>. Ticks are considered the main vectors and reservoir of several pathogenic *Rickettsia* and so, vertebrate hosts are necessary to perpetuate infection cycles and disperse vectors and associated agents<sup>1,6,10</sup>. As mentioned, rickettsial pathogens have been found in bat ticks,

particularly from the spotted fever group (SFG), but associated infections in these mammals are poorly studied<sup>3-5,7</sup>. Serological surveys in Brazil and Georgia provided evidence for the presence of antibodies against antigens of several members of *Rickettsia* from the SFG (*i.e.*, *R. rickettsii*, *R. parkeri*, *R. amblyommii*, *R. rhipicephali*, *R. conorii*, and *R. bellii*) in blood samples collected from insectivorous bats roosting in urban areas<sup>14,15</sup>. Bacteraemia in bats from urban and suburban areas have also been found (*R. africae*, *R. conorii*, and *Rickettsia* sp.)<sup>5,8,9</sup>. In this study, we report the detection of *Rickettsia* closely related to *R. rickettsii*, *R. felis*, and *R. typhi* in spleen samples from nine Mexican fruit bats (*A. jamaicensis*) based on sequence data of two conserved genes (Table). To our knowledge, this is the first report of *R. felis* and *R. typhi*-like *Rickettsia* in bats. The results from this study are important because of the zoonotic potential of these bacteria since there are many human cases in Mexico, particularly in Yucatan, that have been attributed to close contacts with ectoparasitic vectors and its reservoirs (dogs, cats, and opossums) in rural and urban environments<sup>10</sup>. Further studies are needed to investigate potential transmission

cycles involving bats. Ectoparasites were not found on the captured bats, however, these vectors spend up to 90 per cent of their lives off-host<sup>5,16</sup>. Moreover, the social grooming behaviour of bats could contribute to decrease the ectoparasite load<sup>4,6,16</sup>. The presence of circulating bacteria in the absence of these vectors suggests the possibility that *Rickettsia* sp. can maintain a replicative cycle in these mammals having scope to study the reservoir capabilities of these animals<sup>3</sup>. Although nine of the 22 samples were positive for rickettsial DNA, our study was based on a small sample size of bats which was a limitation. Future studies should increase the number of samples and actively involve investigations in ectoparasites in a broader region. Another limitation was the short distance between the mist nets and the ground during the capture, which could have had an impact on the species distribution of the captured bats.

Overall, this study suggests that surveillance and monitoring of rickettsiosis cases should include bats, mainly in regions with close animal-human contacts because there is a possible involvement of these mammals in the ecologic dynamics of *Rickettsia* transmission.

**Financial support & sponsorship:** This study was supported by a grant (CONACYT-251053) from the National Council of Science and Technology, Mexico.

**Conflicts of Interest:** None.

**César Lugo-Caballero<sup>1</sup>, Marco Torres-Castro<sup>1</sup>,  
Karina López-Ávila<sup>1</sup>,  
Silvia Hernández-Betancourt<sup>2</sup>, Henry Noh-Pech<sup>1</sup>,  
Raúl Tello-Martín<sup>1</sup>, Fernando Puerto-Manzano<sup>1</sup> &  
Karla Dzul-Rosado<sup>1,\*</sup>**

<sup>1</sup>Emerging & Re-emerging Diseases Laboratory,  
Regional Research Center “Hideyo Noguchi” &  
<sup>2</sup>Department of Zoology, Campus of Biological &  
Agricultural Sciences, Autonomous University of  
Yucatan, Merida, Yucatan, México

\*For correspondence:  
karla.dzul@correo.uady.mx

Received June 19, 2019

## References

- Alvarez-Hernandez G, Roldan JFG, Milan NSH, Lash RR, Behravesh CB, Paddock CD. Rocky Mountain spotted fever in Mexico: past, present, and future. *Lancet Infect Dis* 2017; 17 : e189-96.
- Álvarez-López DI, Ochoa-Mora E, Heitman KN, Binder AM, Álvarez-Hernández G, Armstrong PA. Epidemiology and clinical features of rocky mountain spotted fever from enhanced surveillance, Sonora, Mexico: 2015-2018. *Am J Trop Med Hyg* 2021; 104 : 190.
- Kuo CC, Lin YF, Yao CT, Shih HC, Chung LH, Liao HC, *et al.* Tick-borne pathogens in ticks collected from birds in Taiwan. *Parasites and Vectors* 2017; 10 : 587.
- Mühldorfer K. Bats and bacterial pathogens: A review. *Zoonoses Public Health* 2013; 60 : 93-103.
- Reeves WK, Beck J, Orlova M V, Daly JL, Pippin K, Revan F, *et al.* Ecology of bats, their ectoparasites, and associated pathogens on saint kitts island. *J Med Entomol* 2016; 53 : 1218-25.
- Hornok S, Szöke K, Meli ML, Sándor AD, Görföl T, Estók P, *et al.* Molecular detection of vector-borne bacteria in bat ticks (Acari: Ixodidae, Argasidae) from eight countries of the Old and New Worlds. *Parasit Vectors* 2019; 12 : 50.
- Sanchez-Montes S, Guzman-Cornejo C, Martinez-Najera Y, Becker I, Venzal JM, Labruna MB. *Rickettsia lusitaniae* associated with *Ornithodoros yumatensis* (Acari: Argasidae) from two caves in Yucatan, Mexico. *Ticks Tick Borne Dis* 2016; 7 : 1097-101.
- Dietrich M, Tjale MA, Weyer J, Kearney T, Seamark EC, Nel LH, *et al.* Diversity of *Bartonella* and *Rickettsia* spp. in bats and their blood-feeding ectoparasites from south Africa and Swaziland. *PLoS One* 2016; 11 : e0152077.
- Cicuttin GL, De Salvo MN, La Rosa I, Dohmen FEG. *Neorickettsia risticii*, *Rickettsia* sp. and *Bartonella* sp. in *Tadarida brasiliensis* bats from Buenos Aires, Argentina. *Comp Immunol Microbiol Infect Dis* 2017; 52 : 1-5.
- Dzul-Rosado KR, Mendez N, Lugo-Caballero C, Zavala-Castro JE, Gomez-Carro S. Epidemiologic profile and clinical course of four confirmed rickettsiosis cases in Southern Mexico during 2016. *Clin Case Rep* 2018; 6 : 119-24.
- Medellín RA, Arita H, Sánchez O. Identificación de los murciélagos de México: Clave de Campo. 2nd ed. México DF: Instituto de Ecología, Universidad Nacional Autónoma de México; 2008 . p.79.
- Choi YJ, Jang WJ, Kim JH, Ryu JS, Lee SH, Park KH, *et al.* Spotted fever group and typhus group rickettsioses in humans, South Korea. *Emerg Infect Dis* 2005; 11 : 237-44.
- Blair PJ, Jiang J, Schoeler GB, Moron C, Anaya E, Cespedes M, *et al.* Characterization of spotted fever group *rickettsiae* in flea and tick specimens from northern Peru. *J Clin Microbiol* 2004; 42 : 4961-7.
- Reeves WK, Streicker DG, Loftis AD, Dasch GA. Serologic survey of *Eptesicus fuscus* from Georgia, U.S.A. for *Rickettsia* and *Borrelia* and laboratory transmission of a *Rickettsia* by bat ticks. *J Vector Ecol* 2006; 31 : 386-9.
- D’Auria SRN, Camargo MCGO, Pacheco RC, Savani ESM, Dias MAG, da Rosa AR, *et al.* Serologic Survey for Rickettsiosis in Bats from São Paulo City, Brazil. *Vector-Borne Zoonotic Dis* 2010; 10 : 459-63.
- Ter Hofstede HM, Fenton MB. Relationships between roost preferences, ectoparasite density, and grooming behaviour of neotropical bats. *J Zool* 2005; 266 : 333-40.