Rickettsioses are a zoonoses characterized by fever and exanthematic syndrome and has become an important public health problem in Mexico\(^1\). 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\(^1,2\). 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\(^1\). 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\(^3\). 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\(^3-5\). There are few reports that describe a *Rickettsia*-associated bacteraemia in bats, but little is known about its implications in zoonotic transmission\(^3-9\). 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*\(^7\). 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\(^1,10\). Somatic measurements, age (juvenile or adult), species, and sex were recorded\(^11\). 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\(^10\).

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)\(^12\). For PCR detection of the 17 kDa lipoprotein antigen (*htrA*) gene, primers FwRr1175F and Rr2608R were used\(^13\). 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*...
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).

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

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

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. Several studies investigated the role of bats in the transmission of infectious agents, including vector-borne bacteria such as Bartonella, Anaplasma, and Borrelia. 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. 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. 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. amblyomma, R. rhipicephali, R. conorii, and R. bellii) in blood samples collected from insectivorous bats roosting in urban areas. Bacteraemia in bats from urban and suburban areas have also been found (R. africae, R. conorii, and Rickettsia sp.). 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.
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. 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. Although none 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.

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