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*Original Article*

# Bats are a potential reservoir of pathogenic *Leptospira* species in Colombia

Jose Mateus[1], Natalia Gómez[1], María Teresa Herrera-Sepúlveda[1,2], Marylin Hidalgo[1], Jairo Pérez-Torres[2], Claudia Cuervo[1]

*[1] Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia*
*[2] Unidad de Ecología y Sistemática (UNESIS). Laboratorio de Ecología Funciona, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia*

## Abstract

Introduction: Bats have become an epidemiologically significant source of pathogenic microorganisms, such as leptospires, the causative agents of leptospirosis. However, little information exists about bats and their potential role as a reservoir of pathogenic *Leptospira* spp. in Colombia. The aim of this study was to evaluate the presence of pathogenic *Leptospira* spp. in the kidneys of bats from the Caribbean region of Colombia deposited in the collection of mammals of the Museo Javeriano de Historia Natural (MPUJ-MAMM).

Methodology: DNA was extracted from twenty-six kidney samples from a total of 13 species of bats captured in Colombia. First, 16S ribosomal RNA conventional PCR was performed to detect the presence of *Leptospira* spp. Then, in samples that tested positive, LipL32 PCR was performed to detect pathogenic *Leptospira* spp. by sequencing and phylogenetic analysis.

Results: The presence of *Leptospira* spp. was observed in 7/26 (26.9%) bats from the following 6 species: *Carollia perspicillata, Glossophaga soricina*, *Dermanura phaeotis*, *Uroderma bilobatum*, *Desmodus rotundus,* and *Lophostoma silvicolum,* and pathogenic *Leptospira* spp. were detected in 4/26 samples (15.4%).

Conclusions: This study suggests that bats present in the Caribbean region of Colombia could be potential reservoirs of pathogenic *Leptospira* spp.

**Key words:** *Leptospira* spp.; bats; zoonotic disease; leptospirosis.

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## Introduction

Leptospirosis, a bacterial zoonotic disease with a worldwide distribution, is caused by infection with pathogenic spirochetes of the genus *Leptospira* and affects humans and domestic and wild animals [1]. This disease is a public health problem that affects 0.5 to 1 million people per year, with a mortality rate ranging from 5 to 10%. Leptospirosis is more prevalent in urban slum-dwellers and subsistence farmers in developing countries that are located in tropical and subtropical areas [2,3]. Spirochetes reside in the kidneys of infected mammals and can cause infections that vary in severity from asymptomatic to acute infection in humans and animals. *Leptospira* spp. are mainly transmitted by contact with infected animal urine (direct) or urine-contaminated environments (mud or water; indirect) via mucosal membranes or open skin wounds [4]. The disease may be underestimated because it is often misdiagnosed as other flu-like febrile illnesses [5]. However, due to hematogenous spreading, the bacteria may cause other more severe complications, such as Weil's syndrome, meningoencephalitis or pulmonary hemorrhage [4].

*Leptospira* spp. are part of a diverse bacterial complex that includes pathogenic, intermediate, and saprophytic or nonpathogenic species. The bacteria can be classified into serovars, which are commonly defined as groups of *Leptospira* species with a specific serological variety; approximately 300 serovars have been identified, including 200 pathogenic serovars of epidemiological importance [1], and pathogenic and nonpathogenic serovars occur within several species [6]. In Colombia, although few studies have evaluated the prevalence of *Leptospira* infection in animals and humans, it has been found that humans can acquire pathogenic serovars by contact with infected animals or contaminated water, suggesting that it is important to identify the major serovars of *Leptospira* spp. and their reservoirs to help prevent the spread of the disease by implementing public health intervention measures [7,8].

Different mammals have been identified in the transmission cycle of leptospirosis, such as wild, peridomestic and insectivorous rodents, as well as other wild and domestic animals. Rodents and dogs are often identified as potential sources of human infection, but other animals, such as cattle, birds, reptiles and bats, have also been suggested as reservoirs of pathogenic *Leptospira* spp. [9,10]. Bats, which are mammals of the order Chiroptera, are involved in many biological functions within an ecosystem (seed dispersal, pollination activity and pest control). Due to some of their life history traits, such as high mobility, broad distribution and social behavior, the relevance of bats as reservoirs of zoonotic pathogens has been highlighted [11]. Bats are one of the most diverse and abundant groups of mammal species in Neotropical ecosystems. These animals represent approximately 20% of the mammal species in the world, and approximately 205 species have been described in Colombia, corresponding to 36% of the bat species in the world [12]. Bats are natural reservoirs for viral, protozoal, fungal, and bacterial human pathogens, such as the rabies virus, trypanosomes and leptospires [13]. It was recently shown that tropical regions with a high richness of bat species are infected by pathogenic *Leptospira* spp. [14]. For instance, a diverse group of leptospires, including pathogenic (*L. interrogans*, *L. kirschneri*, and *L. borgpetersenii*) and intermediate (*L. fainei*) species, was detected in the kidneys of bats from the Peruvian Amazon, suggesting that bats are a reservoir of *Leptospira* species [15]. However, little information is available about bats and their potential role as a reservoir of pathogenic *Leptospira* spp. in Colombia.

Herein, we evaluated the presence of *Leptospira* spp. in the kidneys of bats from the Caribbean region in Colombia deposited in the collection of mammals of the Museo Javeriano de Historia Natural (MPUJ-MAMM).

## Methodology

*Bat samples*

The bat samples (kidneys of bats) were obtained from the collection of mammals at the MPUJ-MAMM. The requirements to use samples from the collection were completed according to the MPUJ-MAMM. The bats were captured in tropical dry forest areas of the Caribbean region of Colombia as described previously [16]. Kidney samples from each bat were stored in 70% ethanol at 4 ºC prior to DNA extraction. The Research and Ethics Committees of the Pontificia Universidad Javeriana approved this study.

*Detection of Leptospira spp.*

Genomic DNA was extracted from bat kidney samples using a High Pure PCR Template Preparation kit according to the manufacturer's instructions (Roche, Mannheim, Germany). Afterwards, to evaluate the DNA integrity and to rule out the presence of inhibitors in the sample, conventional PCR was performed with the CytB Uni forward (5'-TCATCMTGATGAAAYTTYGG-3') and CytB Uni reverse (5'-ACTGGYTGDCCBCCRATTCA-3') primers, which amplify cytochrome B from small mammals, according to previously reported procedures [17]. Then, to detect DNA from the genus *Leptospira* in samples that tested positive for CytB by PCR, PCR was performed with the Lep 1 (5'-GGCGGCGCGTCTTAAACATG-3') and Lep 2 (5'-TTCCCCCCATTGAGCAAGATT-3') primers, which amplify a 331 bp fragment of the 16S ribosomal RNA (rRNA) gene from *L. interrogans* serovar Canicola, using previously described PCR conditions [18]. For both types of PCR, the following controls were included: reaction (water added in the room containing the reaction mixture), gray (water added in the room where the sample was added to the reaction) and positive (genomic DNA from *Leptospira* spp.). The reaction products were resolved using 1% agarose gel electrophoresis followed by staining with the SYBR™ Safe DNA Gel Stain (Invitrogen, Waltham, MA, USA).

*Detection of pathogenic leptospires*

To detect DNA from pathogenic *Leptospira* spp., PCR was performed with the LipL32-270F (5'-CGCTGAAATGGGAGTTCGTATGATT-3') and LipL32-692R5-CCAACAGATGCAACGAAAGATCCTTT-3') primers, which amplify the gene for the major outer-membrane lipoprotein LipL32, which is an important virulence factor present in all pathogenic strains of *Leptospira* spp. [19,20]. As mentioned above, the reaction, gray and positive controls were included, and each conventional PCR was repeated at least twice for reproducibility. PCR products were visualized using 1% agarose gel electrophoresis, followed by staining with the SYBR Safe DNA Gel Stain (Invitrogen).

*Phylogenetic analysis*

Samples that tested positive in 16S rRNA and LipL32 conventional PCR were further identified by sequencing analyses. The amplicons were purified using a Wizard® DNA Clean-Up System kit (Promega, Madison, WI, USA) and then sequenced in both directions using a 3500 Genetic Analyzer (Applied

Biosystems, Foster City, CA, USA). The forward and reverse sequences obtained in the present study were assembled, edited and compared among themselves and with reference sequences available in GenBank after alignment using the Clustal algorithm. A phylogenetic analysis was performed using the maximum likelihood (ML) method based on the Kimura 2-parameter model [21], and 1000 bootstrap replicates were performed using the complete deletion option and the Close-Neighbor-Interchange algorithm of the MEGA software, Version 6 [22].

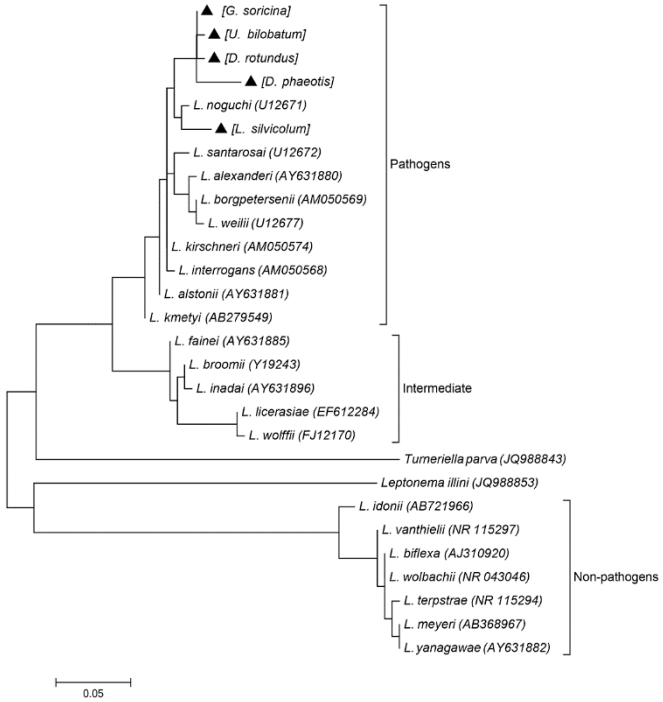
## Results

*Detection of Leptospira spp.*

A total of 26 bat kidney samples from 11 genera and 12 species were analyzed. The analyzed samples of each species and their corresponding feeding habits are summarized in Table 1. *Leptospira* spp. were observed in 7 of 26 (26.9%) bats from the following 6 species: *C. perspicillata, G. soricina*, *D. phaeotis*, *U. bilobatum*, *D. rotundus,* and *L. silvicolum.* Interestingly, bats with *Leptospira* spp. had different feeding habits, which included frugivorous, insectivorous, nectarivorous, and hematophagous habits (Table 1).

To analyze the phylogenetic classification of the *Leptospira* spp. into pathogenic, intermediate, and nonpathogenic groups, a phylogenetic analysis of 16S rRNA genes was performed. Alignment of the *Leptospira* sequences from bats captured in this region

**Figure 1.** Phylogenetic analysis via maximum likelihood (ML) analyses of the 16S rRNA genes of *Leptospira* spp.




The tree with the highest log likelihood is shown and is drawn to scale, with branch lengths representing the number of substitutions per site. The sequences retrieved in this study are indicated by black triangles followed by the species name of the bat, and the GenBank numbers from the reference sequences are indicated in brackets. The *Leptospira* spp. and their groups are listed to the right of each branch.

**Table 1.** Bat species from the Caribbean region in Colombia, their ecological data and *Leptospira* spp. Detection.

| Bat species | Feeding habits | Municipality† | Number of samples | *Leptospira* spp. detection | | Frequency¶ (%) |
|---|---|---|---|---|---|---|
| | | | | 16S rRNA§ | LipL32§ | |
| *Artibeus planirostris* | Frugivorous | Los Córdoba and Buenavista | 6 | ND | ND | 0.0 |
| *Carollia perspicillata* | Frugivorous | Los Córdoba, Canalete and Buenavista | 5 | 1 | 1 | 20.0 |
| *Glossophaga soricina* | Nectarivorous (occasionally feeds on pollen, fruits and insects) | Los Córdoba and Canalete | 3 | 2 | 1 | 66.6 |
| *Dermanura phaeotis* | Frugivorous | Canalete and Buenavista | 2 | 1 | ND | 50.0 |
| *Uroderma bilobatum* | Frugivorous, insectivorous or nectarivorous | Buenavista | 2 | 1 | 1 | 50.0 |
| *Noctilio albiventris* | Carnivorous, insectivorous | Los Córdoba | 2 | ND | ND | 0.0 |
| *Carollia castanea* | Frugivorous | Los Córdoba | 1 | ND | ND | 0.0 |
| *Desmodus rotundus* | Hematophagous | Los Córdoba | 1 | 1 | 1 | 100.0 |
| *Lophostoma silvicolum* | Insectivorous | Buenavista | 1 | 1 | ND | 100.0 |
| *Molossus molossus* | Insectivorous | Buenavista | 1 | ND | ND | 0.0 |
| *Saccopteryx leptura* | Insectivorous | Buenavista | 1 | ND | ND | 0.0 |
| *Vampyriscus nymphaea* | Frugivorous | Buenavista | 1 | ND | ND | 0.0 |
| | **Total** | | **26** | **7/26** | **4/7** | **26.9** |

†Corresponds to the site where the bats were captured; §16S rRNA and LipL32 PCR was performed as described in the Materials and Methods; ¶The frequency was determined from the results obtained by 16S rRNA PCR; ND, not detectable by PCR.

of Colombia showed an overall identity of 92.0 - 96.7% among the sequences. In addition, these sequences had identity values of 95.5%, 88.6%, and 72.4% with the pathogenic, intermediate, and nonpathogenic leptospire sequences reported in GenBank, respectively. ML analysis of the 16S rRNA genes generated a tree that showed a clear clustering of the *Leptospira* sequences into three distinct branches, as previously described [6]: (1) pathogenic species, (2) intermediate species and (3) nonpathogenic species. In particular, the bat *Leptospira* sequences clustered with the pathogenic species (Figure 1).

*Detection of pathogenic leptospires*

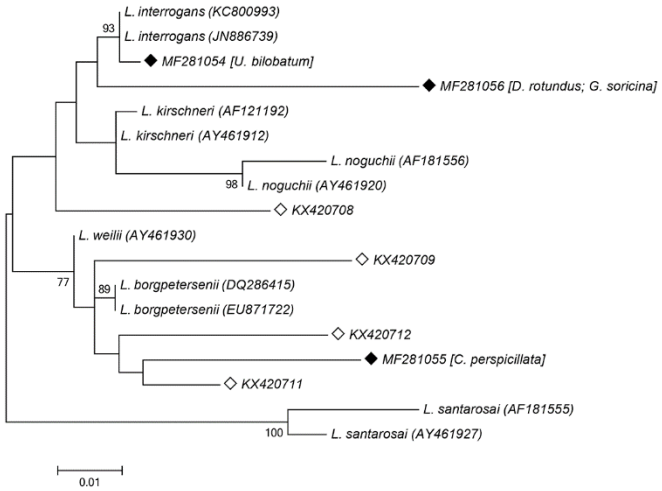
Amplification of the LipL32 gene was observed in 4 of 26 kidney samples (15.4%) (Table 1). The sequences isolated from *D. rotundus* and *G. soricina* were identical (GenBank accession number MF281056), and they showed identity values of 94.6% and 90.7% with the sequences isolated from *U. bilobatum* (GenBank accession number MF281054) and *C. perspicillata* (GenBank accession number MF281055), respectively. The identity between the sequences isolated from *U. bilobatum* and *C. perspicillata* was 93.6%. As shown in Figure 2, these sequences were close to those of some *Leptospira* spp. that are known zoonotic pathogens. The LipL32 sequence obtained from *C. perspicillata* (MF281055) was close to that obtained from the *L. borgpetersenii* clade, and it had an identity of 95.9% with the reference sequences. The sequence isolated from *G. soricina* and *D. rotundus* (MF281056) and the sequence from *U. bilobatum* (MF281054) were closest to the sequence of the *L. interrogans* clade; these sequences had genetic identity values of 94.9% and 99.6% with the *L. interrogans* reference sequences, respectively. In addition, the *Leptospira* sequence obtained in the present study from *C. perspicillata* was related to the variants KX420711 and KX420712 (from *Myotis blythii* and *Miniopterus schreibersii*, respectively), detected in bats from Georgia (USA), and was similar to that of *L. borgpetersenii* [23]. Thus, these results suggest that the sequences obtained from LipL32 gene amplification in kidney samples from bats corresponded to the pathogenic group of *Leptospira*.

## Discussion

Leptospirosis is a bacterial zoonotic disease caused by infection with spirochetes known as *Leptospira* spp. In humans, the disease has been associated with socioeconomically disadvantaged populations and it has been reported as an infection associated with adventure traveling [3,24]. *Leptospira* spp. have been detected in domestic and wild animals, such as rodents, canines, cattle, pigs, sheep, sea lions, marsupials, and bats [4,10]. Recently, several studies have highlighted the role of bats as potential reservoirs of different pathogenic microorganisms, including *Leptospira* spp. [13]. A wider variety of bats has been described in Colombia than in the rest of Latin America [25]. However, to our knowledge, there are currently no reports of the presence of *Leptospira* spp. in this group of small mammals in Colombia. Thus, to evaluate the presence of these bacteria in bats in Colombia, we detected pathogenic *Leptospira* spp. in the kidneys of different species of bats from the Caribbean region that were deposited in the MPUJ-MAMM.

In this study, *Leptospira* spp. were detected in 7 of 26 (26.92%) bats, including 6 species with different feeding habits. This spirochete has been detected in bats from Latin American countries, such as Brazil and Peru, with a prevalence of 2% and 3.4%, respectively [15,18]. However, a study in the United States did not detect the presence of *Leptospira* spp. in bats [26], which suggests that the prevalence of these bacteria in bat populations may vary according to the region of study. Interestingly, in the present work, the presence of these bacteria was detected in *D. phaeotis* and *L. silvicolum*, in which the presence of *Leptospira* spp. had not been detected previously in bats from America [14,27].

**Figure 2.** Phylogenetic analysis via ML analyses of the lipL32 genes of *Leptospira* spp.




The tree with the highest log likelihood is shown, and ML bootstrap values greater than 70 are indicated at each node. The tree is drawn to scale, with branch lengths representing the number of substitutions per site. The sequences retrieved in this study are indicated by black rhombuses with the GenBank number followed by the bat species name. The GenBank numbers from the reference sequences are indicated in brackets, and the sequences of *Leptospira* spp. obtained from bat species in previous studies are indicated by white rhombuses.

Moreover, it is estimated that the presence of *Leptospira* spp. has been detected in approximately 50 species of bats with different feeding habits [14,28]. In this study, *Leptospira* spp. were detected in different species of bats independent of their feeding habits. Recently, it was shown that bats from Southern Brazil exhibit no associations among the frequency of positive results for pathogenic *Leptospira* and the age, sex, species, season of collection, location, or feeding habits of the bats [28]; this observation is similar to the results obtained in the present study. However, these results may be related to the number of bats analyzed in the sample used in the present work.

In Colombia, different studies have been carried out at the local level to determine the prevalence of leptospirosis in humans in different tropical areas. According to the National Health Institute of Colombia, in 2005, the prevalence of leptospirosis was 13.1% in a group of butchers and rice workers in the Caribbean region in Colombia [29]. However, a recent study showed that in this department, the circulation and transmission of different serovars of *L. interrogans* occur between animals (pigs and dogs), humans and the environment, and it has been described that, in this same region, the prevalence of leptospirosis in humans was 67.9% in 2013, with a significant increase in human leptospirosis cases compared to the results reported in 2005 [7,8]. In this study, the presence of *Leptospira* spp. was detected in bats from this same region, which suggests that bats could be involved in the transmission cycle of the disease and that it is important to study the role of bats in the epidemiology of leptospirosis in this region. This work is the first to detect pathogenic species of *Leptospira* spp. in populations of bats from the Caribbean region of Colombia, and we highlight the importance of evaluating the species of bats that are dominant at the local level [30] since, due to their abundance, they may have a greater probability of contacting humans, persisting in transformed environments in the Caribbean region and occupying the homes of people [31].

To date, approximately 22 species of *Leptospira* have been described and are classified according to their pathogenicity, as follows: pathogenic, intermediate (with low pathogenicity), and saprophytic or nonpathogenic species (free-living bacteria that are found in the microenvironment and are generally considered not to infect animals) [1]. Although it has been commonly described that the pathogenic *Leptospira* spp. have similar degrees of pathogenicity, a recent phylogenomic analysis identified 4 subgroups of pathogenic leptospires, which could explain the degree of virulence of each species [32]. Interestingly, in the present study, analysis of 16S rRNA conventional PCR amplicons by sequencing and phylogeny showed that these leptospires detected in bats from the tropical dry forests of the Caribbean region of Colombia are grouped in the pathogenic group of *Leptospira* spp. In addition, using the same methodological strategy mentioned above, the sequences identified by LipL32 conventional PCR amplification showed a high identity with those of the most pathogenic *Leptospira* spp., as described previously [20].

Thus, the findings of the present work suggest that bats present in tropical dry forests of the Caribbean region of Colombia could be potential reservoirs of pathogenic *Leptospira* spp.

## Conclusions

Bats from the Caribbean region of Colombia harbor pathogenic *Leptospira* spp. These preliminary data show that bats could play an important role in maintaining these bacteria in nature. These data also reinforce the need for increased efforts to understand the roles of wild animals in the maintenance, spread, and transmission of zoonotic microorganisms and, therefore, to propose measures for their prevention and control. However, it remains necessary to also consider the irreplaceable ecological (seed dispersal and pollination), economic (insect pest control) and public health (control of insects that transmit diseases to people) benefits that bats offer.

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## References

1. Picardeau M (2017) Virulence of the zoonotic agent of leptospirosis: still terra incognita? Nat Rev Microbiol 15: 297-307.
2. Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, Stein C, Abela-Ridder B, Ko AI (2015) Global morbidity and mortality of leptospirosis: A systematic review. PLoS Negl Trop Dis 9: 1-19.
3. World Health Organization (2010) Report of the first meeting of the leptospirosis burden epidemiology reference group. Available: http://apps.who.int/iris/bitstream/handle/10665/44382/9789241599894_eng.pdf?sequence=1. Accessed: 4 May 2018.

4. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM, Peru-United States Leptospirosis C (2003) Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis 3: 757-771.
5. Haake DA, Levett PN (2015) Leptospirosis in humans. Curr Top Microbiol Immunol 387: 65-97.
6. Levett PN (2014) Systematics of leptospiraceae. In Adler B, editor. *Leptospira* and Leptospirosis. Berlin, Heidelberg: Springer: 11-20.
7. Calderon A, Rodriguez V, Mattar S, Arrieta G (2014) Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. Trop Anim Health Prod 46: 427-432.
8. Rodríguez-Barreto H, Lozano C, Bedoya C, Grondona L (2009) Prevalence of Leptospirosis in humans from urban area of the municipality of Puerto Libertador, Córdoba, Colombia. RIAA 1: 23-28. [Article in Spanish].
9. Barragan V, Chiriboga J, Miller E, Olivas S, Birdsell D, Hepp C, Hornstra H, Schupp JM, Morales M, Gonzalez M, Reyes S, de la Cruz C, Keim P, Hartskeerl R, Trueba G, Pearson T (2016) High leptospira diversity in animals and humans complicates the search for common reservoirs of human disease in rural Ecuador. PLoS Negl Trop Dis 10: 1-14.
10. Vieira AS, Pinto PS, Lilenbaum W (2018) A systematic review of leptospirosis on wild animals in Latin America. Trop Anim Health Prod 50: 229-238.
11. Kunz TH, Braun de Torrez E, Bauer D, Lobova T, Fleming TH (2011) Ecosystem services provided by bats. Ann N Y Acad Sci 1223: 1-38.
12. Ramírez-Chaves HE, Suárez-Castro AF, González-Maya JF (2016) Recent changes to the list of mammals in Colombia. Mammalogy Notes 1: 1-9. [Article in Spanish].
13. Allocati N, Petrucci AG, Di Giovanni P, Masulli M, Di Ilio C, De Laurenzi V (2016) Bat-man disease transmission: zoonotic pathogens from wildlife reservoirs to human populations. Cell Death Discov 2: 16048.
14. Dietrich M, Muhldorfer K, Tortosa P, Markotter W (2015) *Leptospira* and bats: Story of an emerging friendship. PLoS Pathog 11: 1-6.
15. Matthias MA, Diaz MM, Campos KJ, Calderon M, Willig MR, Pacheco V, Gotuzzo E, Gilman RH, Vinetz JM (2005) Diversity of bat-associated *Leptospira* in the Peruvian Amazon inferred by bayesian phylogenetic analysis of 16S ribosomal DNA sequences. Am J Trop Med Hyg 73: 964-974.
16. Ballesteros J, Pérez-Torres J (2015) Effect of Silvopastoral and conventional extensive livestock management on the assembly of bats associated with fragments of tropical dry forest in Córdoba, Colombia. Available: https://repository.javeriana.edu.co/handle/10554/19650. Accessed 20 October 2017. [Document in Spanish].
17. Schlegel M, Ali HS, Stieger N, Groschup MH, Wolf R, Ulrich RG (2012) Molecular identification of small mammal species using novel cytochrome B gene-derived degenerated primers. Biochem Genet 50: 440-447.
18. Bessa TA, Spichler A, Chapola EG, Husch AC, de Almeida MF, Sodre MM, Savani ES, Sacramento DR, Vinetz JM (2010) The contribution of bats to leptospirosis transmission in Sao Paulo City, Brazil. Am J Trop Med Hyg 82: 315-317.
19. Yang CW, Wu MS, Pan MJ, Hsieh WJ, Vandewalle A, Huang CC (2002) The *Leptospira* outer membrane protein LipL32 induces tubulointerstitial nephritis-mediated gene expression in mouse proximal tubule cells. J Am Soc Nephrol 13: 2037-2045.
20. Levett PN, Morey RE, Galloway RL, Turner DE, Steigerwalt AG, Mayer LW (2005) Detection of pathogenic leptospires by real-time quantitative PCR. J Med Microbiol 54: 45-49.
21. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.
22. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725-2729.
23. Bai Y, Urushadze L, Osikowicz L, McKee C, Kuzmin I, Kandaurov A, Babuadze G, Natradze I, Imnadze P, Kosoy M (2017) Molecular survey of bacterial zoonotic agents in bats from the country of Georgia (Caucasus). PLoS One 12: 1-12.
24. Gundacker ND, Rolfe RJ, Rodriguez JM (2017) Infections associated with adventure travel: A systematic review. Travel Med Infect Dis 16: 3-10.
25. Mickleburgh SP, Hutson AM, Racey PA (2002) A review of the global conservation status of bats. Oryx 36: 18-34.
26. Harkin KR, Hays M, Davis R, Moore M (2014) Use of PCR to identify *Leptospira* in kidneys of big brown bats (*Eptesicus fuscus*) in Kansas and Nebraska, USA. J Wildl Dis 50: 651-654.
27. Ballados-Gonzalez GG, Sanchez-Montes S, Romero-Salas D, Colunga Salas P, Gutierrez-Molina R, Leon-Paniagua L, Becker I, Mendez-Ojeda ML, Barrientos-Salcedo C, Serna-Lagunes R, Cruz-Romero A (2018) Detection of pathogenic *Leptospira* species associated with phyllostomid bats (Mammalia: *Chiroptera*) from Veracruz, Mexico. Transbound Emerg Dis 65: 773-781.
28. Mayer FQ, Dos Reis EM, Bezerra AVA, Cerva C, Rosa J, Cibulski SP, Lima FES, Pacheco SM, Rodrigues RO (2017) Pathogenic *Leptospira* spp. in bats: Molecular investigation in Southern Brazil. Comp Immunol Microbiol Infect Dis 52: 14-18.
29. National Institute of Health of Colombia (2009) Report of the Leptospirosis protocol. Available: https://www.minsalud.gov.co/Documentos y Publicaciones/PROTOCOLO DE LEPTOSPIROSIS.pdf. Accessed 4 May 2018. [Document in Spanish].
30. Ríos-Blanco MC, Pérez-Torres J (2015) Diet of the dominant species of the frugivorous bat assemblage in a tropical dry forest (Colombia). Mastozoología Neotropical 22: 103-111. [Article in Spanish].
31. Vela-Vargas IM, Pérez-Torres J (2012) Bats associated with remnants of tropical dry forest in an extensive livestock system (Colombia). Chiroptera Neotropical 18: 1089-1100. [Article in Spanish].
32. Xu Y, Zhu Y, Wang Y, Chang YF, Zhang Y, Jiang X, Zhuang X, Zhu Y, Zhang J, Zeng L, Yang M, Li S, Wang S, Ye Q, Xin X, Zhao G, Zheng H, Guo X, Wang J (2016) Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic *Leptospira*. Sci Rep 6: 1-11.

## Corresponding author

Claudia Cuervo
Grupo de Enfermedades Infecciosas, Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá D.C. - Colombia, Carrera. 7 # 43-82, Edificio 50 (Félix Restrepo) Oficina 608.
Tel.: +5713208320, Ext. 4153,
Fax: +5713208320, Ext. 4021,
Email: claudia.cuervo@javeriana.edu.co

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