

# Leptospirosis Infecting Bats

Subjects: **Microbiology**

Contributor: Fernando P Monroy , Sergio Solari , Juan Álvaro Lopez , Piedad Agudelo-Flórez , Ronald Guillermo Peláez-Sanchez

Leptospirosis is a globally distributed zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*. This zoonotic disease affects humans, domestic animals, and wild animals. Colombia is considered an endemic country for leptospirosis, and Antioquia is the second department in Colombia, with the highest number of reported leptospirosis cases.

Leptospira

bats

Colombia

leptospirosis

species

type

## 1. Introduction

Leptospirosis is an emerging zoonotic disease caused by pathogenic bacteria of the genus *Leptospira* <sup>[1]</sup>. Previous studies have estimated that 1.03 million cases and 58,900 deaths occur due to leptospirosis worldwide annually <sup>[2]</sup>. Leptospirosis is considered a neglected disease, found mainly in the tropical regions of developing countries <sup>[3]</sup> and is now recognized as an emerging infectious disease due to large outbreaks in different regions of the world, which are associated with environmental disasters, and extreme climate change. In many endemic regions, severe forms of the disease, such as Weil's disease and pulmonary hemorrhage syndrome have emerged as the leading cause of death <sup>[4]</sup>. Currently, about 65 genomic *Leptospira* species have been identified (NCBI database: <https://www.ncbi.nlm.nih.gov/genome> [accessed on 30 April 2021]), which are subdivided into four main clades according to the phylogenetic analysis of 1371 conserved genes: pathogens (P1), pathogens (P2), saprophytes (S1), and saprophytes (S2) <sup>[4][5]</sup>. Through serological classification about 300 *Leptospira* serovars have been described, which are grouped into approximately 30 serogroups and about 200 of these serovars have been considered pathogenic <sup>[6]</sup>. Colombia is an endemic country for leptospirosis with at least 500 cases every year <sup>[7]</sup>. Antioquia is the second department in Colombia with the highest number of confirmed cases of leptospirosis <sup>[7]</sup>, with a seroprevalence close to 12.5% <sup>[8]</sup>. *Leptospira interrogans* and *L. santarosai* have been identified as the causative agents of this disease <sup>[9]</sup>. Therefore, this department is an important region in Colombia for the study of leptospirosis.

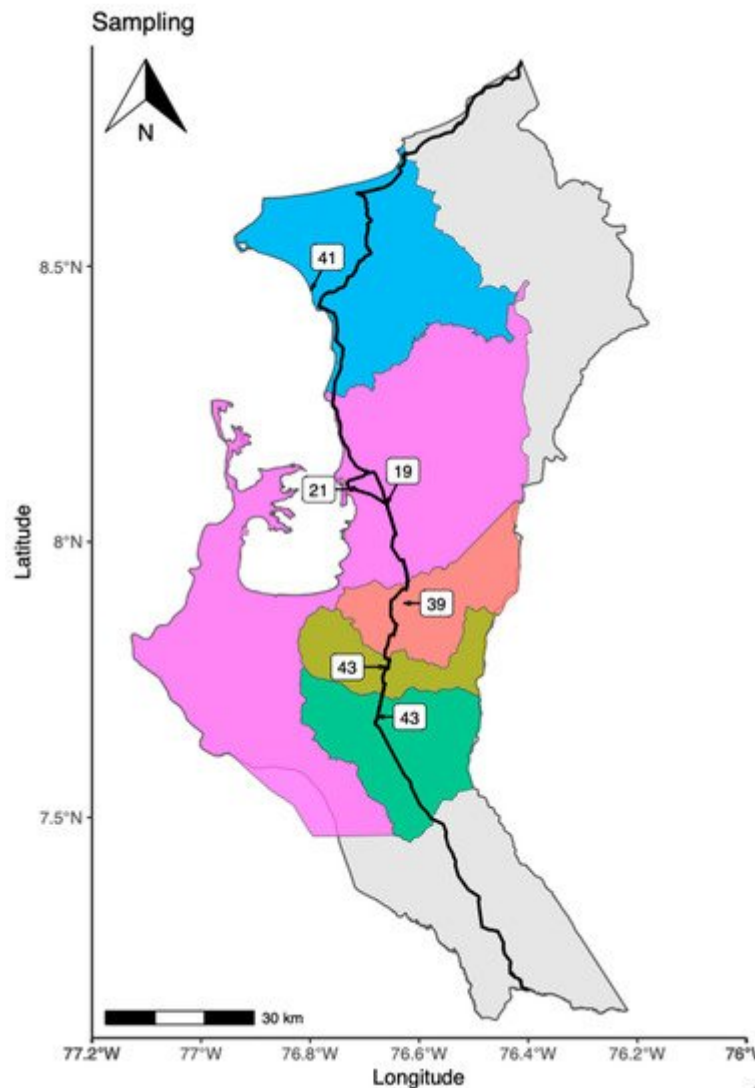
Rodents and dogs are often identified as potential sources of human infection, but other mammals have also been identified in the transmission cycle of leptospirosis <sup>[1]</sup>. Globally, various studies have explored the biological role of bats as reservoirs of zoonotic pathogens due to their ability to fly long distances and disperse pathogens (viruses <sup>[10]</sup>, bacteria <sup>[11]</sup>, parasites <sup>[12]</sup> and fungi <sup>[13]</sup>) through urine, saliva, and feces. Bats are the only true flying mammals, belonging to the order Chiroptera <sup>[14]</sup>. This order includes over 1400 different species in 21 families <sup>[15]</sup>, which are scattered throughout the world, except Antarctica <sup>[16]</sup>. These mammals are oriented and hunt by

echolocation [17]. Depending on the species they can feed on arthropods, fruits, pollen, fish, blood, and other vertebrates (carnivores) [10]. Some species can hibernate [18], form large colonies [19], migrate long distances [20], and have long lifespans (approximately 35 years) [21].

Bats have been identified worldwide as an important reservoir of different *Leptospira* species (*L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, *L. fainei*) and their role in disease transmission, and spillover in the life cycle of this bacterium has yet to be defined [22]. Currently, more than 50 species of infected bats with *Leptospira* have been reported in different countries, including Peru [23], Brazil [24], Argentina [25], Australia [26], Comoros island and Madagascar [27], Reunion Island [28], Mayotte Island [22], Indonesia [23], Malaysia [24], Tanzania [25], Trinidad [26], Sudan [27], Democratic Republic of Congo [28], Africa [29], and Azerbaijan [30]. In Colombia, two studies have reported the presence of bats naturally infected with *Leptospira* [31][32]. Due to the above characteristics, bats could act as excellent spillover of *Leptospira* species to the environment, favoring contamination of water and soil, serving as a direct or indirect source of infection for other animals, which are the main reservoirs and disseminators of the bacteria. The objective of the present investigation was to detect *Leptospira* species infecting different bat species in the Urabá region (Antioquia-Colombia) and to evaluate the genetic diversity of the circulating *Leptospira* species. This information will illustrate the role of bats in the transmission cycle of human leptospirosis.

## 2. Places of Bat's Capture

The investigation was carried out in the Urabá region (Antioquia-Colombia). The sampling was undertaken in five different municipalities (Chigorodó—43 captured bats), (Carepa—43 captured bats), (Apartadó—39 captured bats), (Turbo—40 captured bats), (Necoclí—41 captured bats). In total, 206 bats were captured. The map of the Urabá region and the exact location of the three sampling sites are shown in **Figure 1**.

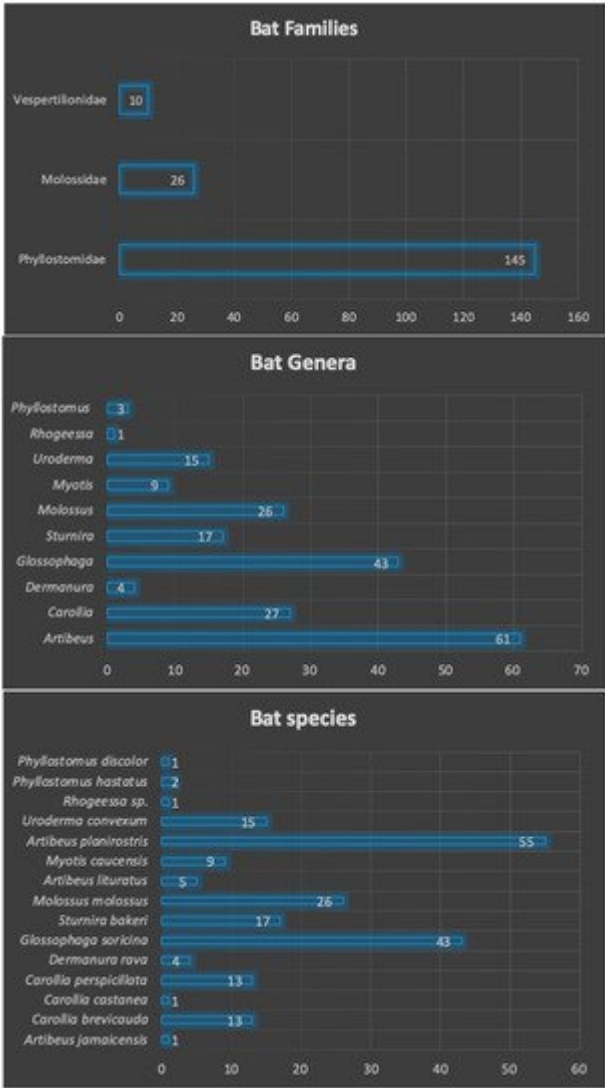


**Figure 1.** The geographical location of capture sites. The map shows the geographical location of the five municipalities that were used to capture the 206 bats (blue-Necoclí, purple-Turbo, orange-Apartadó, dark green-Carepa and light green-Chigorodó). These capture sites are located in the Urabá region (Antioquia-Colombia). The map was generated using the environment and programming language R and packages (ggplot2, MappingGIS, sfMaps, spData, ggrepel, ggspatial, cowplot).

### 3. Families, Genera and Species of Captured Bats

Researchers captured 206 bats in the five municipalities of the Urabá region (Antioquia, Colombia). These bats were classified into three different families (Phyllostomidae, Molossidae, and Vespertilionidae), 10 different genera (*Artibeus*, *Carollia*, *Dermanura*, *Glossophaga*, *Sturnira*, *Molossus*, *Myotis*, *Uroderma*, *Rhogeessa*, *Phyllostomus*), and fifteen different species (*Artibeus jamaicensis*, *A. lituratus*, *A. planirostris*, *Carollia brevicauda*, *C. castanea*, *C. perspicillata*, *Dermanura rava*, *Glossophaga soricina*, *Sturnira bakeri*, *Molossus molossus*, *Myotis caucensis*, *Uroderma convexum*, *Phyllostomus hastatus*, *P. discolor*), and one unidentified species belonging to the genus

*Rhogeessa*. The genera, families and species are shown in **Figure 2**. These species have different feeding habits, such as frugivorous (60.19%), insectivores (17.47%), omnivore (1.45%), nectarivores (20.87%) (**Table 1**).



**Figure 2.** Diversity and abundance of bats captured in the five municipalities of the Urabá region. **Figure 2** shows the 3 families, 10 genera, and 15 species of bats that were captured in the five sampling areas. The number of individuals for each taxonomic group classification are also indicated.

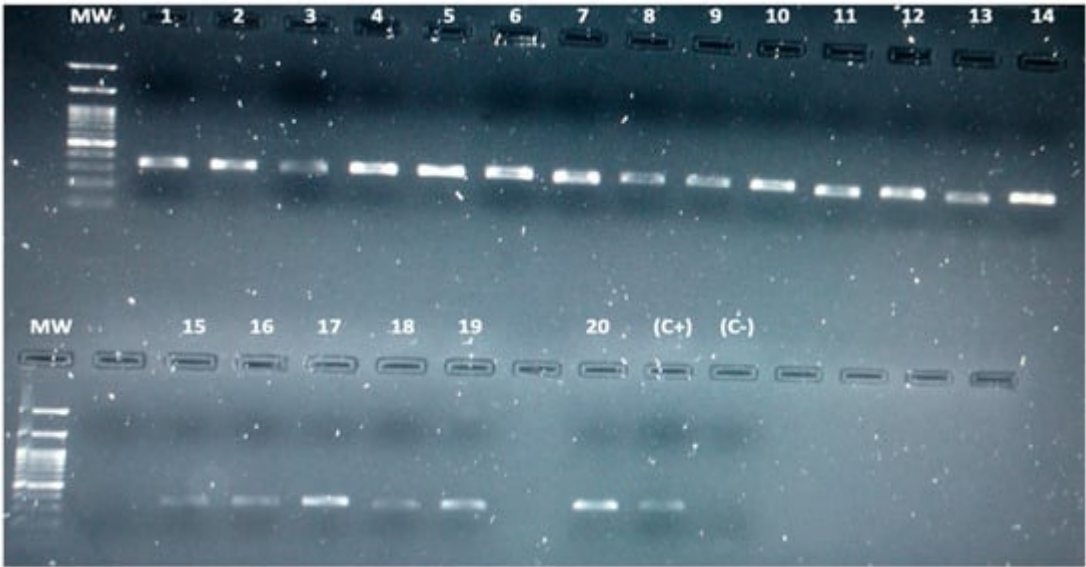
**Table 1.** Diversity of bats captured in the study. This table shows information about the species, number, percentage, frequency and feeding habits of the 206 bats captured.

Species	Number	Percentage (%)	Frequency	Feeding Habits
Artibeus jamaicensis	1	0.49%	0.005	frugivore
Carollia brevicauda	13	6.31%	0.063	frugivore
Carollia castanea	1	0.49%	0.005	frugivore

Species	Number	Percentage (%)	Frequency	Feeding Habits
<i>Carollia perspicillata</i>	13	6.31%	0.063	frugivore
<i>Dermanura rava</i>	4	1.94%	0.019	frugivore
<i>Glossophaga soricina</i>	43	20.87%	0.209	nectarivore
<i>Sturnira bakeri</i>	17	8.25%	0.083	frugivore
<i>Molossus molossus</i>	26	12.62%	0.126	insectivore
<i>Artibeus lituratus</i>	5	2.43%	0.024	frugivore
<i>Myotis caucensis</i>	9	4.37%	0.044	insectivore
<i>Artibeus planirostris</i>	55	26.70%	0.267	frugivore
<i>Uroderma convexum</i>	15	7.28%	0.073	frugivore
<i>Rhogeessa</i> sp.	1	0.49%	0.005	insectivore
<i>Phyllostomus hastatus</i>	2	0.97%	0.010	omnivore
<i>Phyllostomus discolor</i>	1	0.49%	0.005	omnivore
TOTAL	206	100%	1	

**PCR**  
*Leptospira*

spp. Twenty individual bats were positive for *Leptospira* (20/206), obtaining a 9.7% of infected bats (**Figure 3**). Positive bats for *Leptospira* infection were found in the 5 municipalities studied (Chigorodó: 3 bats, Carepa: 2 bats, Apartadó: 3 bats, Turbo: 10 bats, and Necoclí: 2 bats). Additionally, 6 different species of bats were found to be infected: *Carollia perspicillata*, *Dermanura rava*, *Glossophaga soricina*, *Molossus molossus*, *Artibeus planirostris*, and *Uroderma convexum*. According to sex, 11 males (55%) and 9 females (45%) were found infected. Regarding feeding habits, 12 frugivores (60%), 6 nectarivores (30%), and 2 insectivores (10%) bats were found infected (**Table 2**).



**Figure 3.** Molecular detection of bats naturally infected with *Leptospira*. The figure shows a 1% agarose gel with the amplification products of 20 bats infected with *Leptospira* spp. The band (331 base pair) corresponding to a fragment of the 16S ribosomal gene. A 100 base pair molecular weight markers were used. Additionally, a positive control (C+: *Leptospira interrogans*) and a negative control (C-: PCR reagents without DNA) were used in all reactions.

**Table 2.** Natural infection of bats with different *Leptospira* species. This table shows the code of the positive samples, *Leptospira* species identified by amplification of the 16S ribosomal gene, bat species infected, and the municipality from which the sampling area originated.

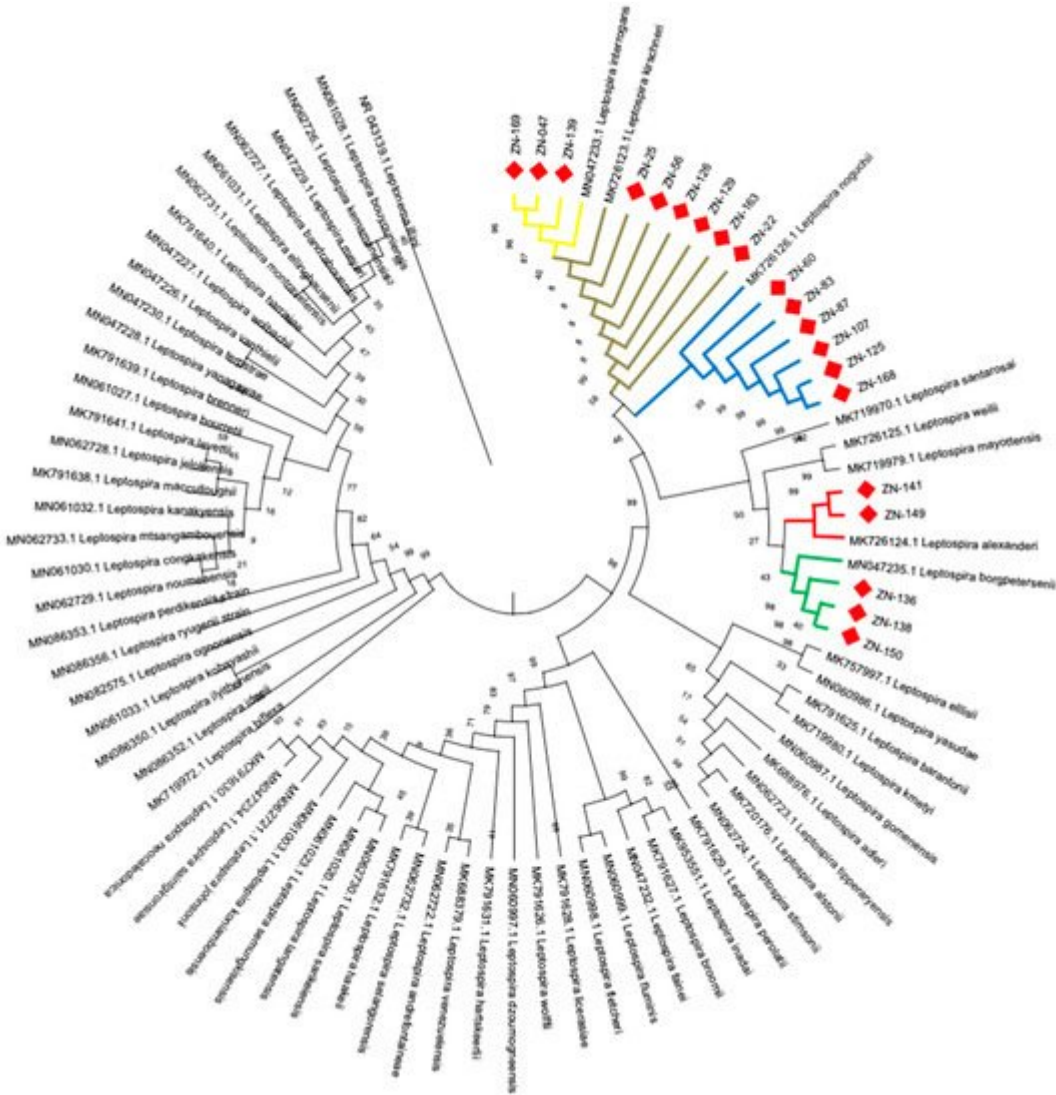
Code	Phylogenetic Identification (16S Ribosomal Gene)	Infected Species	Feeding Habits	Gender	Municipality
ZM-022	<i>Leptospira kirschneri</i>	<i>Carollia perspicillata</i>	Frugivore	Female	Carepa
ZM-025	<i>Leptospira kirschneri</i>	<i>Dermanura rava</i>	Frugivore	Male	Carepa
ZM-047	<i>Leptospira interrogans</i>	<i>Glossophaga soricina</i>	Nectarivore	Female	Apartadó
ZM-056	<i>Leptospira kirschneri</i>	<i>Glossophaga soricina</i>	Nectarivore	Male	Apartadó
ZM-060	<i>Leptospira noguchii</i>	<i>Glossophaga soricina</i>	Nectarivore	Female	Apartadó
ZN-083	<i>Leptospira noguchii</i>	<i>Uroderma convexum</i>	Frugivore	Male	Chigorodó
ZN-087	<i>Leptospira noguchii</i>	<i>Uroderma convexum</i>	Frugivore	Male	Chigorodó
ZN-107	<i>Leptospira noguchii</i>	<i>Uroderma convexum</i>	Frugivore	Female	Chigorodó
ZN-125	<i>Leptospira noguchii</i>	<i>Molossus molossus</i>	Insectivore	Female	Turbo
ZN-126	<i>Leptospira kirschneri</i>	<i>Molossus molossus</i>	Insectivore	Male	Turbo
ZN-129	<i>Leptospira kirschneri</i>	<i>Artibeus planirostris</i>	Frugivore	Male	Turbo
ZN-136	<i>Leptospira borgpetersenii</i>	<i>Glossophaga soricina</i>	Nectarivore	Female	Turbo
ZN-138	<i>Leptospira borgpetersenii</i>	<i>Glossophaga soricina</i>	Nectarivore	Female	Turbo
ZN-139	<i>Leptospira interrogans</i>	<i>Artibeus planirostris</i>	Frugivore	Male	Turbo
ZN-141	<i>Leptospira alexanderi</i>	<i>Uroderma convexum</i>	Frugivore	Female	Turbo
ZN-149	<i>Leptospira alexanderi</i>	<i>Glossophaga soricina</i>	Nectarivore	Male	Turbo
ZN-150	<i>Leptospira borgpetersenii</i>	<i>Artibeus planirostris</i>	Frugivore	Male	Turbo
ZN-163	<i>Leptospira kirschneri</i>	<i>Artibeus planirostris</i>	Frugivore	Male	Turbo
ZN-168	<i>Leptospira noguchii</i>	<i>Uroderma convexum</i>	Frugivore	Male	Necoclí

## 5. Identification of Leptospira Species by Phylogenetic Analysis



Code	Phylogenetic Identification (16S Ribosomal Gene)	Infected Species	Feeding Habits	Gender	Municipality	following
ZN-169	<i>Leptospira interrogans</i>	<i>Uroderma convexum</i>	Frugivore	Female	Necoclí	20–10%), 20–30%).

Results of the phylogenetic identification are shown in **Figure 4**.



**Figure 4.** Identification of *Leptospira* species infecting bats by phylogenetic analysis of the 16S ribosomal gene. Phylogenetic reconstruction of the 16S ribosomal gene of the genus *Leptospira* is shown. Red diamonds represent the bats infected with *Leptospira* spp. *Leptospira borgpetersenii*, *Leptospira alexanderi*, *Leptospira noguchii*, *Leptospira interrogans*, and *Leptospira kirschneri* were the *Leptospira* species found infecting this bat population.

## 6. Host-Pathogen Relationship between Bats and *Leptospira*

The host-pathogen association is as follows: *Leptospira borgpetersenii* infected 2 bats species (*Glossophaga soricina* and *Artibeus planirostris*), *Leptospira alexanderi* infected 2 bats species (*Uroderma convexum* and *Glossophaga soricina*), *Leptospira noguchii* infected 3 bats species (*Glossophaga soricina*, *Uroderma convexum*, and *Molossus molossus*), *Leptospira interrogans* infected 3 bats species (*Glossophaga soricina*, *Artibeus*

*planirostris*, and *Uroderma convexum*) and *Leptospira kirschneri* infected 5 bats species (*Carollia perspicillata*, *Dermanura rava*, *Glossophaga soricina*, *Molossus molossus*, and *Artibeus planirostris*). The number of infected bats for each *Leptospira* species is shown in **Table 3**. Additionally, no renal infection was detected in 9 bat species (*A. jamaicensis*, *C. brevicauda*, *C. castanea*, *S. bakeri*, *A. lituratus*, *M. caucensis*, *P. hastatus*, *P. discolor*, and *Rhogeessa* sp.).

**Table 3.** Natural infection of bats with different *Leptospira* species. The table shows the host-pathogen relationship between 6 *Leptospira* species and 6 bats species susceptible to infection. The number of bats infected by each *Leptospira* species is shown in parentheses.

Leptospira Species	Infected Bat Species	Infected Bats
Leptospira borgpetersenii	Glossophaga soricina (2)	3
	Artibeus planirostris (1)	
Leptospira alexanderi	Uroderma convexum (1)	2
	Glossophaga soricina (1)	
Leptospira noguchii	Glossophaga soricina (1)	6
	Uroderma convexum (4)	
	Molossus molossus (1)	
Leptospira interrogans	Glossophaga soricina (1)	3
	Artibeus planirostris (1)	
	Uroderma convexum (1)	
Leptospira kirschneri	Carollia perspicillata (1)	6
	Dermanura rava (1)	
	Glossophaga soricina (1)	
	Molossus molossus (1)	
	Artibeus planirostris (2)	



- ## 7. Conclusion

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