World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Epidemiology and molecular study of *Leptospira spp*. in bats and rodents in the Republic of Guinea

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World Journal of Advanced Research and Reviews, 2023, 19(03), 391–403

Publication history: Received on 26 July 2023; revised on 04 September 2023; accepted on 06 September 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.19.3.1790

Abstract

Introduction: Leptospirosis is a zooanthroponosis caused by spirochete bacteria called leptospire of the genus Leptospira.

Objective: To contribute to the knowledge and circulation of the Leptospira germ in humans in the Republic of Guinea.

Methods: This prospective and descriptive cross-sectional study lasted 18 months, from June 2019 to December 2020.

Results: 687 samples, including 388 bat kidney tissues and 299 kidney tissues from captured rodents, were analysed at IRBAG's Guineo-Russian laboratory. The molecular diagnostic technique (RT-qPCR) and sequencing were carried out. Among rodents, 15 species were captured in the various administrative regions of Guinea: *Mastomys natalensis, Mastomys erythroleucus. Mastomys sp, Praomys daltoni, Praomys rostratus, Praomys sp, Rattus rattus, Mus musculus, Mus musculoides, Mus sp, Lemmiscomys striatus, Gerbilliscus guineae, Cricetomys gambianus, Crocidura olivieri, Crocidura sp.* The N'zérékoré region recorded the highest number of captures 226 (87 *Mastomys natalensis* ,61 *Mus musculus,* 46 *Crocidura sp.* 26 *Rattus rattus,* 5 *Crocidura olivieri,* 1 *Mus sp.*), followed by the Kindia region with 66 cases (25 *Rattus rattus* ,18 *Mastomys sp,* 10 *Mastomys erythroleucus*, 6 *Mus musculus,* 2 *Praomys daltoni,* 2 *Praomys sp,* 2 *Mus musculoides,* 1 *Praomys rostratus,* 1 *Lemmiscomys striatus,* 1 *Gerbilliscus guineae,* 1 *Cricetomys gambianus),* the Faranah region with 4 cases (3 *Mus musculoides,*1 *Mastomys sp.).* The Mamou, Boké and Labé regions recorded no cases. Sixteen species of bats were captured and tested: *Rousettus aegyptiacus, Lissonycteris angolensis, Epomophorus gambianus, Hipposideros jonesi, Hipposideros ruber, Hipposideros abae, Hipposideros sp., Rhinolophus sp., Chaerephon sp.., Chaerephon major, Chaerephon pumilus, Mops condylurus, Mops sp., Nycteris sp., Scotophilus leucogaster, Miniopterus sp*. The Kindia region recorded the highest number of positive carriers 184 (102 *Hipposideros ruber*, 14 *Lissonycteris angolensis*, 13 *Hipposideros jonesi*,13 *Mops condylurus*,12 *Hipposideros sp*., 8 *Rousettus aegyptiacus*,8 *Chaerephon pumilus*, 5 *Hipposideros abae*,3 *Scotophilus leucogaster*,3 *Miniopterus sp.*,2 *Rhinolophus sp*.,1 *Mops sp.*), the N'Zérékoré region 134 cases (1 *Lissonycteris angolensis*, 1 *Chaerephon sp.*,41 *Chaerephon major*, 6 *Chaerephon pumilus*, 94 *Mops condylurus*,1 *Mops sp*,1 *Nycteris sp.*),the Boké region 52 cases (50 *Hipposideros ruber*, 2 *Rhinolophus sp.*),the Faranah region 7 cases (7 *Epomophorus gambianus*), the Mamou and Labé regions recorded 0 cases each. *Mastomys sp.* 3/17, *Praomys daltoni* 0/2, *Praomys rostratus* 1/1, *Rattus rattus* 1/13, *Mus musculus* 0/5, *Mus musculoides* 0/0, *Crocidura olivieri*), the Nzérékoré region with 4 positive cases out of 129. (*Mastomys natalensis* 1/37, *Rattus rattus* 1/14, *Crocidura sp*.2/30), the Faranah region recorded 0 positive cases out of a total of 4 (*Mastomyssp.* 0/1, *Mus musculoides* 0/3) and the Boké region 0 cases out of a total of 0. *Mastomus natalensis*, number of specimens 56 including 14 positive cases, i.e. 6.9%; *P.daltoni*, number of specimens 3 including 2 positive cases, i.e. 1.43%; *Praomus sp*, number of specimens 7 including 0 positive cases; *Rattus rattus*, number of specimens 75 including 1 positive case, i.e. 0.48%; *Mus musculus*, number of

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specimens 3 including 0 positive cases; *P.fallax,* number of specimens 1 including 0 positive cases; *L.sikapusi,* number of specimens 1 including 0 positive cases; *Crocidura sp,* number of specimens 2 of which 0 positive; *S.leucogaster,* number of specimens 32 of which 0 positive; *T. thersites,* number of specimens 23 of which 0 positive; *N.hispida*, number of specimens 4 of which 0 positive; *E.helvum,* number of specimens 2 of which 0 positive. Sequencing yielded fragments of the LiP32 gene were isolated from all positive samples. Analysis established the identity of the samples with the *L. interrogans* genome.

Conclusion: The results obtained prove that the leptospirosis pathogen does circulate in the Republic of Guinea in both rodents and bats.

Keywords: *Leptospira spp*: *Leptospira spp*; Rodents; Bats; RT-qPCR; Guinea

1. Introduction

Leptospirosis is a cosmopolitan zoono-anthroponosis caused by filamentous bacteria called Leptospire of the genus Leptospira. It attacks both humans and animals. It is a major public health problem. It affects almost a million people a year worldwide, including 60,000 deaths, but these figures are probably underestimated due to the lack of specificity of symptoms and the difficulty of using diagnostic techniques. Leptospirosis is endemic in countries with tropical climates, and climate change may increase its incidence [1]. It has been described all over the world, including in temperate countries. Conditions are very favorable for the circulation of leptospires in several tropical countries such as Asia, Latin America and Sub-Saharan Africa [1]. Worldwide incidence estimates put the number of cases at 1.03 million per year, giving an average incidence rate of 14 cases per 100,000 inhabitants and a mortality rate of 7%. These figures are underestimated due to a lack of information for certain areas of the world with no diagnostic facilities [2]. The areas most affected are those with tropical or subtropical climatic conditions, where the incidence varies from 13 to 150 cases per 100,000 inhabitants per year. The incidence appears to be higher in areas with a low standard of living, with 2 categories of group particularly at risk in developing countries: people working in agriculture, and people living in insalubrious conditions. Leptospirosis affects 80% of men, half of whom are in the 20-50 age bracket [3]. Leptospirosis is caused by spirochete bacteria called leptospire of the genus Leptospira. It attacks humans as well as domestic animals (cattle, pigs, dogs) and wild animals (rodents), which excrete the bacteria in their urine [4]. The pathogenic species, *Leptospira interrogans*, comprises more than 200 serovars grouped into twenty serogroups [5]. The usual symptoms vary from one individual to another, ranging from a simple fever to multiple organ failure, with mortality in 5 to 10% of cases. These bacteria are aerobic and are not resistant to dryness or hypertonicity, but can tolerate alkalinisation down to a pH of 7.8 [6]. Global warming, heavy rainfall, increasing urbanisation, etc. are all factors in the spread of leptospire. People living in unhealthy urban or peri-urban habitats and subsistence farmers are particularly at risk. The bacteria are transmitted naturally from domestic or wild vertebrate animals (e.g. rodents) to humans, and vice versa, who excrete them in their urine [7]. In sub-Saharan Africa, many countries have climatic characteristics conducive to the development and transmission of leptospires, but the incidence and prevalence remain difficult to assess. However, Gabon has been reporting cases regularly since 1994 [8]. In Senegal, 3.1% of blood samples were found to be positive for leptospirosis in blood donors and a prevalence of 14.3% of canine leptospirosis in the Kaolack region, with serovar *L.icterohemorragiae* dominating over serovar Pomona, with 69% of positive cases compared with 23 [9]. In Nigeria, surveys of healthy populations showed leptospirosis seroprevalence in the Eastern and Plateau states, at 14% and 18% respectively [10].

2. Materials and working methods

2.1. Study environment and setting

Our study was carried out in the Republic of Guinea. The Guineo-Russian laboratory of the Guinea's Applied Biology Research Institute (IRBAG) provided the setting for the study.

2.2. Working method

This is a cross-sectional, descriptive and analytical prospective study which lasted 18 months, from June 2019 to December 2020. It focused on three types of population, namely: Patients received in consultation in a febrile state in various hospitals in Guinea; Bats captured in Guinea and Rodents captured in Guinea. Sampling was simple random and the size of the samples (n=687) from the two populations was obtained using the SCHWARTZ formula: 388 kidney tissues from bats and 299 kidney tissues from rodents. All rodents and bats caught in our traps during the survey period were included in our study.

2.3. Bio-material

Our bio-material consisted of rodent kidney tissue and bat kidney tissue.

2.4. Study variables

2.4.1. Biological variables

- RT-qPCR
- Species
- Genotypes.

2.4.2. Epidemiological variables

- Sampling regions
- Sex and gender

2.5. Data collection and computer analysis

The data were compiled manually, entered into Word 2007 and processed and analysed using Excel and SPSS version 21.

2.6. Limitations and difficulties of the study

Small sample size for rodents and bats (due to COVID-19), storage of blood samples in health facilities and transport to IRBAG.

3. Results and Discussion

The study of the detection of Leptospira spp. 16s RNA in rodents, bats and sequencing yielded results in the form of tables, which were interpreted, commented on and discussed according to the available literature data.

3.1. Distribution of rodent capture results

Table 1 Results of rodent (*Rodentia* and *Eulipotyphla*) captures for *leptospira* in different administrative regions.

Species	Natural regions						Total
	Kindia	Nzérékoré	Boké	Labé	Mamou	Faranah	
Mastomys natalensis	-	87	-	-	-	-	87
Mastomys erythroleucus	10	-	-	-	-	-	10
Mastomys spp.	18	-	-	-	-	1	19
Praomys daltoni	2	-	-	-	-	-	2
Praomys rostratus	1	-	-	-	-	-	1
Praomys sp	2	-	-	-	-	-	2
Rattus rattus	25	26	-	-	-	-	51
Mus musculus	6	61	-	-	-	-	67
Mus musculoides	2	-	-	-	-	3	5
Mus sp	0	1	-	-	-	-	1
Lemmiscomys striatus	1	-	-	-	-	-	1
Gerbilliscus guineae	1	-	-	-	-	-	-
Cricetomys gambianus	1	-	-	-	-	-	1
Crocidura olivieri	-	5	-	-	-	-	5

Crocidura sp.	-	46	-	-	-	-	46
Total	69	226	-	-	-	4	299

In this table, we note the presence of 15 species caught in the different natural regions of Guinea which are*: Mastomys natalensis, Mastomys erythroleucus. Mastomys sp, Praomys daltoni, Praomys rostratus, Praomys sp, Rattus rattus, Mus musculus, Mus musculoides, Mus sp, Lemmiscomys striatus, Gerbilliscus guineae, Cricetomys gambianus, Crocidura olivieri, Crocidura sp.*

The N'zérékoré region recorded the highest number of positive results, 226 (87 *Mastomys natalensis*, 61 *Mus musculus*, 46 *Crocidura sp.*, 26 *Rattus rattus*, 5 *Crocidura olivieri*, 1 *Mus sp.*), followed by the Kindia region with 66 cases (25 *Rattus rattus* ,18 *Mastomys sp*, 10 *Mastomys erythroleucus*, 6 *Mus musculus*, 2 *Praomys daltoni*, 2 *Praomys sp*, 2 *Mus musculoides*, 1 *Praomys rostratus*, 1 *Lemmiscomys striatus*, 1 *Gerbilliscus guineae,* 1 *Cricetomys gambianus*), the Faranah region with 4 cases (3 *Mus musculoides*,1 *Mastomys sp.*). The Mamou, Boké and Labé regions each recorded 0 cases.

Table 2 Results of tests for Leptospira in bats in the administrative regions

Species	**Administrative regions**						**Total**
	Kindia	Nzérékoré	Boké	Labé	Mamou	Faranah	
Rousettus aegyptiacus	8	-	-	-	-	-	8
Lissonycteris angolensis	14	1	-	-	-	-	15
Epomophorus gambianus	-	-	-	-	-	7	7
Hipposideros jonesi	13	-	-	-	-	-	13
Hipposideros ruber	102	-	50	-	-	-	152
Hipposideros abae	5	-	-	-	-	-	5
Hipposideros sp.	12	-	-	-	-	-	12
Rhinolophus sp.	2	-	2	-	-	-	4
Chaerephon sp.	-	1	-	-	-	-	1
Chaerephon major	-	41	-	-	-	-	41
Chaerephon pumilus	8	6	-	-	-	-	14
Mops condylurus	13	94	-	-	-	-	107
Mops sp	1	1	-	-	-	-	2
Nycteris sp.	-	1	-	-	-	-	1
Scotophilus leucogaster	3	-	-	-	-	-	3
Miniopterus sp.	3	-	-	-	-	-	3
Total Général	184	145	52	-	-	7	388

From this table, sixteen bat species were captured and tested: *Rousettus aegyptiacus, Lissonycteris angolensis, Epomophorus gambianus, Hipposideros jonesi, Hipposideros ruber, Hipposideros abae, Hipposideros sp., Rhinolophus sp.,*

Chaerephon sp., Chaerephon major, Chaerephon pumilus, Mops condylurus, Mops sp., Nycteris sp., Scotophilus leucogaster, Miniopterus sp.

The Kindia region recorded the highest number of positive cases 184 (102 *Hipposideros ruber*, 14 *Lissonycteris angolensis*, 13 *Hipposideros jonesi*,13 *Mops condylurus*,12 *Hipposideros sp*., 8 *Rousettus aegyptiacus*,8 *Chaerephon pumilus*, 5 *Hipposideros abae*,3 *Scotophilus leucogaster*,3 *Miniopterus sp*.,2 *Rhinolophus sp*.,1 *Mops sp.*),the N'Zérékoré region 134 cases (1 *Lissonycteris angolensis,* 1 *Chaerephon sp.*,41 *Chaerephon major*, 6 *Chaerephon pumilus*,94 *Mops condylurus*,1 *Mops sp*,1 *Nycteris sp.*),the Boké region 52 cases (50 *Hipposideros ruber*, 2 *Rhinolophus sp*.),the Faranah region 7 cases (7 *Epomophorus gambianus*), the Mamou and Labé regions each recorded 0 cases.

In the present study, rRNA for the leptospirosis pathogen was identified in 139 bat species, giving a kidney carriage rate of 22% (30/139). Our study corroborates studies carried out in America by Bunnel JE et al (2000) and Matthias MA et al (2005), which showed that bats, like other mammals, could be reservoirs of Leptospira, particularly because of their abundance and proximity to domestic and wild animals, but also to humans. However, the rate of prevalence of renal carriage observed in our study, 22%, is higher than that found by Matthias et al (2005) who reported a prevalence of renal carriage of 3.4% in Peru. This difference may be explained by the predominance of the humid tropical climate in Guinea, which explains the ability of leptospires to survive in this area for several months after the rainy season [11; 12].

In 2011, Tulsiani et al. demonstrated the existence of potential transmission of leptospires in Australia from fruit bats (*Pteropus sp.)* to rodents via contact between rodents and the urine of excreting bats. It can be assumed that a bat/rodent transmission cycle exists in Guinea, enabling Leptospira to be maintained in environments where these two species cohabit. Consequently, in Guinea, *S. leucogaster, H. jonesi* and *H. ruber* can be considered as maintenance reservoirs and a source of transmission of *Leptospira* [13].

3.2. Results of molecular research on Leptospira spp in the administrative regions.

Leptospira spp DNA was detected in rodents and bats using the PCR method.

Table 3 Detection of Leptospira DNA in rodents (Rodentia and Eulipotyphla) in different regions.

Species	**Administrative regions**				**Total**
	Kindia	Nzérékoré	Boké	Faranah	
Mastomys natalensis	0/0	1/37	0/0	0/0	1/37
Mastomys sp.	3/17	0/0	0/0	0/1	3/18
Praomys daltoni	0/2	0/0	0/0	0/0	0/2
Praomys rostratus	1/1	0/0	0/0	0/0	1/1
Rattus rattus	1/13	1/14	0/0	0/0	2/27
Mus musculus	0/5	0/43	0/0	0/0	0/48
Mus musculoides	0/0	0/0	0/0	0/3	0/3
Crocidura olivieri	0/0	0/5	0/0	0/0	0/5
Crocidura sp.	0/0	2/30	0/0	0/0	2/30
Total	5/38	4/129	0/0	*0/4*	9/171

From this table, we can see that the Kindia region recorded 5 positive cases out of a total of 38 (*Mastomys natalensis* 0/0, *Mastomys sp.* 3/17, *Praomys daltoni* 0/2, *Praomys rostratus* 1/1, *Rattus rattus* 1/13, *Mus musculus* 0/5, *Mus musculoides* 0/0, *Crocidura olivieri*), the N'Zérékoré region 4 positive cases out of 129 (*Mastomys natalensis* 1/37, *Rattus rattus* 1/14, *Crocidura sp.*2 /30), the Faranah region recorded 0 positive cases out of a total of 4 (*Mastomys sp.* 0/1*, Mus musculoides* 0/3) and the Boké region 0 cases out of a total of 0.

The N'Zérékoré region recorded the highest number of samples (129) compared with the Kindia (38) and Faranah (4) regions.

The Kindia region had the highest number of positive samples (5), followed by N'zérékoré (4).

Table 4 Detection of *Leptospira* DNA in bats (*Chiroptera*) in different regions.

Species	**Administrative regions**				**Total**
	Kindia	Nzérékoré	Boké	Faranah	
Rousettus aegyptiacus	6/8	0/0	0/0	0/0	6/8
Lissonnycteris angolensis	5/11	0/1	0/0	0/0	5/12
Hipposideros jonesi	1/4	0/0	0/0	0/0	1/4
Hipposideros ruber	9/39	0/0	0/31	0/0	9/70
Hipposideros sp.	7/12	0/0	0/0	0/0	7/12
Chaerephonn pumilus	0/0	0/1	0/0	0/0	0/1
Mops condylurus	0/0	0/26	0/0	0/0	0/26
Scootophilus leucogaster	0/3	0/0	0/0	0/0	0/3
Miniopterus sp.	2/3	0/0	0/0	0/0	2/3
Total	30/80	0/28	0/31	*0/0*	30/139

In this table, we note that the Kindia region recorded 30 positive cases out of a total of 80 (*Rousettus aegyptiacus* 6/8, *Lissonnycteris angolensis* 5/11, *Hipposideros jonesi* 1/4, *Hipposideros ruber* 9/39, *Hipposideros sp*. 7/12, Chaerephonn pumilus 0/0, *Mops condylurus* 0/0 a, *Scootophilus leucogaster* 0/3, *Miniopterus sp.* 2/3), the N'Zérékoré region 0 positive cases out of 28 (*Rousettus aegyptiacus* 0/0, *Lissonnycteris angolensis* 0/1, *Hipposideros jonesi* 0/0, *Hipposideros ruber* 0/0, *Hipposideros sp.* 0/0, *Chaerephonn pumilus* 0/1, *Mops condylurus* 0/26, *Scootophilus leucogaster* 0/0, *Miniopterus* sp. 0/0), Boké region 0 positive cases out of 31 (*Rousettus aegyptiacus* 0/0, *Lissonnycteris angolensis* 0/0, *Hipposideros jonesi* 0/0, *Hipposideros ruber* 0/31, *Hipposideros sp.* 0/0, *Chaerephonn pumilus* 0/0, *Mops condylurus* 0/0, *Scootophilus leucogaster* 0/0, *Miniopterus sp.* 0/0,), the Faranah region 0 positive cases out of 0 individuals.

Table 5 Results of PCR detection of Leptospira in various species of small mammals in Kindia.

Species	**Localities**	**Number of samples**	**Number of positive cases**	**Percentage**
Mastomus natalensis	Koliady II	35	14	40
	Pastoria	11	2	18,2
	Féréfou II	8	0	0
	Yabara	1	0	0
P.daltoni	Pastoria	3	2	66,7
Praomus sp.	Patoria	5	0	0
	Koliady II	2	0	0
Rattus rattus	Samoria	52	0	0
	Koliady II	8	0	0
	Patoria	4	0	0
	Féréfou II	4	1	25
	Khabya	4	0	0
	Dammakhania	2	0	0

	Daoudaya	1	0	0
Mus musculus	Koliady II	1	0	0
	Yabara	1	0	0
	Khabya	1	0	0
P.fallax	Koliady II	1	0	0
L.sikapusi	Patoria	1	0	0
Crocidura sp.	Patoria	1	0	0
	Khabya	1	0	0
S.leucogaster	Patoria	17	0	0
	Fommèdè	15	0	0
T.thersites	Patoria	12	0	0
	Fommèdè	10	0	0
	Dammakhania	1	0	0
N.hispida	Pastoria	4	0	0
E.helvum	Koliady II	2	0	0

From this table, we note that out of 208 samples of small ruminants, we detected Leptospira RNA in 12 species. The districts Koliady II 14/35 positive cases, i.e. 40%, Pastoria 2/3, i.e. 67%, Féréfou II ¼, i.e. 25%, Samoria 0/52.

Table 6 Carriage of Leptospira spp. in the various rodent species captured.

Nº	**Species**	**Number of captures**	**Number of positive cases**	**% of positive cases**
1	*Mastomus natalensis*	56	14	6.9
2	*P.daltoni*	3	2	1.43
3	*Praomus sp*	7	-	-
4	*Rattus rattus*	75	1	0.48
5	*Mus musculus*	3	-	-
6	*P.fallax*	1	-	-
7	*L.sikapusi*	1	-	-
8	*Crocidura sp.*	2	-	-
9	*S.leucogaster*	32	-	-
10	*T.thersites*	23	-	-
11	*N.hispida*	4	-	-
12	*E.helvum*	2	-	-
Total		209	17	8.13

From this table, we note that *Mastomus natalensis*, number of specimens 56 including 14 positive cases, i.e. 6.9%; *P.daltoni*, number of specimens 3 including 2 positive cases, i.e. 1.43%; *Praomus sp,* number of specimens 7 including 0 positive cases; *Rattus rattus*, number of specimens 75 including 1 positive case, i.e. 0.48%; *Mus musculus*, number of specimens 3 including 0 positive cases; *P.fallax,* number of specimens 1 including 0 positive cases; *L.sikapusi*, number

of specimens 1 including 0 positive cases; *Crocidura sp,* number of specimens 2 of which 0 positive cases; *S.leucogaster,* number of specimens 32 of which 0 positive cases; *T.thersites,* number of specimens 23 of which 0 positive cases; *N.hispida,* number of specimens 4 of which 0 positive cases; *E.helvum,* number of specimens 2 of which 0 positive cases.

The largest number of samples was taken from the species *Rattus rattus* (75), followed by *Mastomus natalensis* (56), *S.leucogaster* (32), *T.thersites*(23), *Praomus sp.* (7), *N.hispida* (4), *Mus musculus* and *P. daltoni* presented the same number of specimens (3), *E.helvum* and *Crocidura sp.* the same number of specimens (2), *P.fallax* and *L.sikapusi* also presented the same number of specimens (1). The highest number of positive specimens was observed in *Mastomus natalensis* (16), *P.daltoni* (2), *Rattus rattus* (1) and *Mus musculus* (3).

Table 7 Detection of *Leptospira* RNA in small ruminants according to capture sites in Kindia.

Places of capture	**Number of animals caught**	**Percentage of positive cases by place of capture**
Pastoria	58	4 (21,1)
Samoria	52	0(0)
Koliady II	49	14 (73,7)
Fommèdè	25	0(0)
Féréfou II	12	1(5,3)
Khabya	6	0(0)
Dammakhania	3	0(0)
Yabara	2	0(0)
Dadia	1	0(0)
Total	208	19 (100)

From this table, we can see that *Leptospira* RNA was detected in 208 small ruminants with a percentage rate of 19%. The highest number of samples was recorded in the Pastoria district (58 samples), followed by the districts of : Samoria (52), Koliady II (49), Fommèdè (25), Féréfou II (12), Khabya (6), Dammakhania (3), Yabara (2) and Dadia (1).The highest number of positive cases was registered in Koliady II,14 positive with a percentage of 73.7%, Pastoria 4 positive with a percentage 21.1% and Féréfou II, 1 case with a percentage 5.3%.

Table 8 Data on collection sites for reservoirs of the leptospirosis agent in Kindia.

N°	**Rodent species caught**	**Capture sites (Biotopes)**
2	*M.natalensis*	Koliady II plantation
4	*M.natalensis*	Koliady II plantation
5	*M.natalensis*	Pastoria, plantation de mange
11	*P.daltoni*	Pastoria, plantation de mange
12	*P.daltoni*	Pastoria, plantation de mange
14	*M.natalensis*	Pastoria
16	*M.natalensis*	Pastoria, plantation de mange
29	*M.natalensis*	Koliady II plantation
36	*M.natalensis*	Koliady II bord de la rivière
40	*M.natalensis*	Koliady II, maison d'habitation
41	*M.natalensis*	Koliady II, maison d'habitation

42	*R.rattus*	Féréfou, maison d'habitation
44	*M.natalensis*	Koliady II, champ de riz
45	*M.natalensis*	Koliady II, champ de riz
46	*M.natalensis*	Koliady II, champ de riz
47	*M.natalensis*	Koliady II, champ de riz
52	*M.natalensis*	Koliady II, bord du marigot
111	*M.natalensis*	Koliady II, champ de riz
204	*M.natalensis*	Koliady II plantation

From this table, we can see that the species *M. natalensis* was the most frequently encountered (736) out of a total of 801 samples, followed by the species *R. rattus* (42) and the species *P. daltoni* (23).

The various samples were taken in dwellings, rice fields, plantations and on the banks of rivers. The largest numbers were taken in houses (near granaries) during the dry season when food is scarce in the plantations.

3.3. Genotyping results for Leptospira spp. encountered in Guinea.

Currently, more than 250 pathogenic *leptospira* serovars have been identified. West African countries are among the most unfavorable for leptospirosis. In Guinea, in previous studies, the circulation of *leptospira spp.* was known, but the species of the pathogen had not yet been identified. The aim of this study was to identify the species of *leptospira spp.* in small mammals (liver and spleen) caught in the Provence region of Kindia. Previously, 5 of the 16 *leptospira* RNA species were detected in these samples by PCR using the LPS test system (FBSI Central Research Institute for Epidemiology, Russia).

Table 9 Genotyping of Leptospira spp. encountered in Guinea.

Nº	**Strains Tested**	**Origin**	**Lip32 Gene**	**Genotype**
1	Strain 1	Rodent	-	*L.interrogans*
2	Strain 2	Rodent	-	*L.interrogans*
3	Strain 3	Rodent	+	*L.interrogans*
4	Strain 4	Rodent	-	*L.interrogans*
5	Strain 5	Rodent	-	*L.interrogans*
6	Strain 6	Rodent	+	*L.interrogans*
7	Strain 7	Rodent	+	*L.interrogans*
8	Strain 8	Rodent	-	*L.interrogans*
9	Strain 9	Rodent	-	*L.interrogans*
10	Strain 10	Rodent	-	*L.interrogans*
11	Strain 11	Rodent	-	*L.interrogans*
12	Strain 12	Rodent	+	*L.interrogans*
13	Souche 13	Rodent	+	*L.interrogans*
14	Souche 14	Rodent	-	*L.interrogans*
15	Souche 15	Rodent	-	*L.interrogans*
16	Souche 16	Rodent	-	*L.interrogans*

For the genetic differentiation of Leptospira from different ecological groups, the detection of the LiP32 gene, which is one of the pathogenicity factors of the pathogen, is recommended. The presence of the desired region was revealed by PCR with specific primers in 5 of the 16 samples. When partial sequencing was performed, fragments of the LiP32 gene were obtained in all positive samples. Analysis using the BLAST algorithm (http. //Blast.ncbi.nlmnihgov) established the identity of the samples with the *L.interrogans* genome. All positive samples were isolated from organs of poly max mice (*Mastomys natalensis*) [11].

Various studies have shown that rodents are resistant to infection by Leptospira and are chronic carriers of the bacteria. We found a prevalence of renal carriage of 5.26%, i.e. 9 cases/171 samples taken. This prevalence is lower than that reported in the literature, with the exception of the study by Collares et al (2000) and Amélie et al (2012), who respectively found prevalences of 85.4% on the island of Terceira and 84.6% on the island of Réunion [12;13; 14;15; 17].

Harper GA et al (2005) demonstrated that the black rat and *R. norvegicus* are omnivorous generalists with great adaptability. In the Philippines and Madagascar, the respective prevalences observed by Villanueva S et al (2014) and Rahelinirina et al (2010), which are 43% and 30.8%, are much higher than the 5.26% observed in the present study [16-18; 20].

Moutou F (1980) showed that house mice live in houses and cultivated fields. The potential importance of mice in the epidemiology of leptospirosis has been demonstrated in many other parts of the world, such as on the island of Terceira (Azores, Portugal) by Collares-Pereira M et al. (2000), Barbados by Mathias MA, Levett PN (2002), Guadeloupe by Michel V (2001) and Pascal M et al. (2004), Argentina by Vanasco NB et al. (2003), Madagascar by Rahelinirina S et al. (2010), Brazil by da Silva EF et al. (2010) [14 ; 19; 21; 22; 25].

The rate of kidney carriage in small mammals in Guinea is 27.8% (32 cases/115), which is very high compared with the rate estimated by Rahelinirina et al. (2010) in Madagascar (9.1%). Our prevalence of 27.8% is significantly lower than that reported in a study conducted on the island of Terceira (Azores) and on Reunion Island, where 85.4% and 84.6% of mice were found to be carriers of leptospires in the kidneys, respectively by Collares-Pereira M (2000) and Desvars A et al. (2012) [14;15;18,26].

We found a difference in the prevalence of kidney carriage observed as a function of age, sex and/or living area in rodents. Our results corroborate those found by Day TD, et al (1997), in certain species living in temperate zones, such as opossums in New Zealand (Trichosurus vulpecula), transmission is dependent on social contacts between individuals and therefore occurs preferentially in adulthood. In contrast, Collares-Pereira M et al (1997) showed that in the Azores, the sex of rodents had no influence on their leptospirosis status, and in *R. rattus* and *R. norvegicus*, age had no influence on seroprevalence or renal carrier status. Nevertheless, the same authors demonstrate that in mice, age has a significant effect on the prevalence of renal carriage, which is higher in adult mice. In their studies, Mohamed-Hassan et al (2010) and Vanasco et al (2003) conclude that the prevalence of leptospirosis is higher in male rats than in females in the Kelantan and Terengganu regions (Malaysia) and in Argentina respectively [14;27;28;24].

According to Blanchard RJ and Blanchard CB (1977), in male or female rats, during fights for territory, dominance or reproduction, the animals make wounds and urinate, so aggressive behaviour is a factor favouring direct transmission of leptospirosis in the rodent population. In rodents, therefore, direct transmission seems to be the predominant mode of transmission compared with indirect contamination. The study by Desvars A, et al (2011), shows that the environment contaminated by rodents in particular is also a source of (indirect) contamination for these species, but above all it represents a source of infection for other animal species (including humans). The rainy season favours the survival of the bacteria in the environment, which is why the incidence of the disease in humans increases at this time of year, particularly in tropical areas [14; 29].

It is important to note that in this study, the largest number of infected animals carrying leptospires among rodents was the species *M. natalensis* (12.31%). Indeed, the circulation of leptospirosis pathogens in *M. natalensis* was described earlier by other authors with a frequency of occurrence of 6% in Tanzania by Mgode G.F et al. (2006) and 0.6% in Africa by Mgode et al. (2015), results that are lower than those found in our study. This rodent species is endemic in African countries south of the Sahara, as reported by Christensen J.T. (1996), from where the species *M. natalensis* can be dangerous to human health [30 ; 31 ; 32].

In 2004, Pascal M et al. showed that cultivated fields act as reservoirs for mice, whereas "savannahs" and tropical rainforests play this role with regard to the black rat; this could justify the wide variation in prevalence observed in our study by species depending on the place of capture [23].

4. Conclusion

Sixteen bat species were obtained: *Rousettus aegyptia-cus, Lissonycteris angolensis, Epomophorus gambianus, Hipposideros jonesi, Hipposideros ruber, Hipposideros abae, Hipposideros sp, Rhinolophus sp., Chaerephon sp., Chaerephon major, Chaerephon pumilus, Mops condylurus, Mops sp., Nycteris sp., Scotophilus leucogaster, Miniopterus sp.*

Among rodents, we captured 15 species in different regions of Guinea: *Mastomys natalensis, Mastomys erythroleucus. Mastomys sp, Praomys daltoni, Praomys rostratus, Praomys sp, Rattus rattus, Mus musculus, Mus musculoides, Mus sp, Lemmiscomys striatus, Gerbilliscus guineae, Cricetomys gambianus, Crocidura olivieri, Crocidura sp.*

For the first time, data has been obtained on the circulation in Guinea of pathogenic *leptospira* belonging to the *L.interrogans* species, which are capable of causing disease in humans and animals.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the managers and technicians of the Russo-Guinean laboratory of IRBAG and those of the Gamal Abdel Nasser University of Conakry for their support in carrying out this work.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

In accordance with international or academic standards, written ethical approval was obtained and retained by the authors.

Statement of informed consent

In accordance with international or academic standards, written parental consent was obtained and retained by the authors.

Authors' contributions

All authors contributed to this work. They have read and approved the final version of the manuscript.

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