

## Article

# Pathogenic *Leptospira* in Commensal Small Mammals from the Extensively Urbanized Coastal Benin

Gualbert Houéménou <sup>1</sup>, Philippe Gauthier <sup>2</sup>, Jonas Etougbehé <sup>1</sup>, Sylvestre Badou <sup>1</sup>, Henri-Joël Dossou <sup>1,3</sup>, David Agossou <sup>1</sup>, Mathieu Picardeau <sup>4</sup> and Gauthier Dobigny <sup>1,2,\*</sup>

<sup>1</sup> École Polytechnique d'Abomey-Calavi, Laboratoire de Recherche en Biologie Appliquée, Unité de Recherche sur les Invasions Biologiques, Université d'Abomey-Calavi, Cotonou 01BP2009, Benin

<sup>2</sup> Institut de Recherche pour le Développement, UMR Centre de Biologie pour la Gestion des Populations (IRD, CIRAD, INRA, Montpellier SupAgro), Montpellier Université d'Excellence, 34988 Montpellier, France

<sup>3</sup> Institut de Géographie, d'Aménagement du Territoire et d'Environnement, Université d'Abomey-Calavi, Cotonou 01BP2009, Benin

<sup>4</sup> Institut Pasteur, Unité Biologie des Spirochêtes, Institut Pasteur, 28 rue du Dr Roux, 75015 Paris, France

\* Correspondence: gauthier.dobigny@ird.fr; Tel.: +229-95463276

Received: 9 July 2019; Accepted: 5 September 2019; Published: 6 September 2019



**Abstract:** Leptospirosis is caused by spirochete bacteria of the genus *Leptospira* that affect one million and kill 60,000 persons annually in the world, who get infected through environmental mammal-excreted (notably rodent) pathogens. Using qPCR and DNA sequencing approaches, we here examine *Leptospira* occurrence and diversity in 971 commensal small mammals in urban and peri-urban habitats from south Benin, where socio-environmental conditions are favorable for human contamination. Prevalence reached 12.9% on average, but showed very important variations in both space and time, thus pointing toward a role of local processes in the maintenance and circulation of rodent-borne leptospires in the area. Prevalence peaks may occur during or one month after moderate (100–200 mm) monthly rainfall, suggesting that rodent-borne leptospires may be more prevalent when standing waters are present, but not at their highest levels (i.e., floods). However, this pattern will have to be confirmed through proper diachronic analysis. Finally, an incomplete but significant host-specificity was observed, with *L. kirschneri* retrieved only in African shrews, and the invasive *Rattus norvegicus* and the native *Mastomys natalensis* preferentially infected by *L. interrogans* and *L. borgpeterseni*, respectively. Our study highlights the urgent need for investigations on human leptospirosis in the extensively urbanized Abidjan–Lagos corridor.

**Keywords:** zoonotic disease; leptospirosis; urbanization; rodents; West Africa

## 1. Introduction

Leptospirosis is caused by spirochete bacteria of the genus *Leptospira* that affect one million and kill 60,000 persons annually in the world [1]. Clinical forms range from asymptomatic cases to renal, hepatic, and/or pulmonary failures that can result in severe syndromes and, ultimately, death (reviewed in Reference [2]). The lack of specific symptoms makes the disease difficult to diagnose; consequently, it may remain unrecognized, especially in developing countries where it is poorly documented and may be easily mistaken for malaria, dengue, yellow fever, hemorrhagic fevers, or pneumonic plague [2–6]. Many pathogenic *Leptospira* species and a wide range of serovars are described (see Reference [7] for a review in Africa). Although many vertebrate species, especially mammals, were found to carry leptospires [8], cattle, pigs, dogs, cats, and rodents are often considered as the main sources of the pathogen for humans who get infected following contact with animal blood, urine, or urine-contaminated waters and soils [2]. As a consequence, leptospirosis is tightly

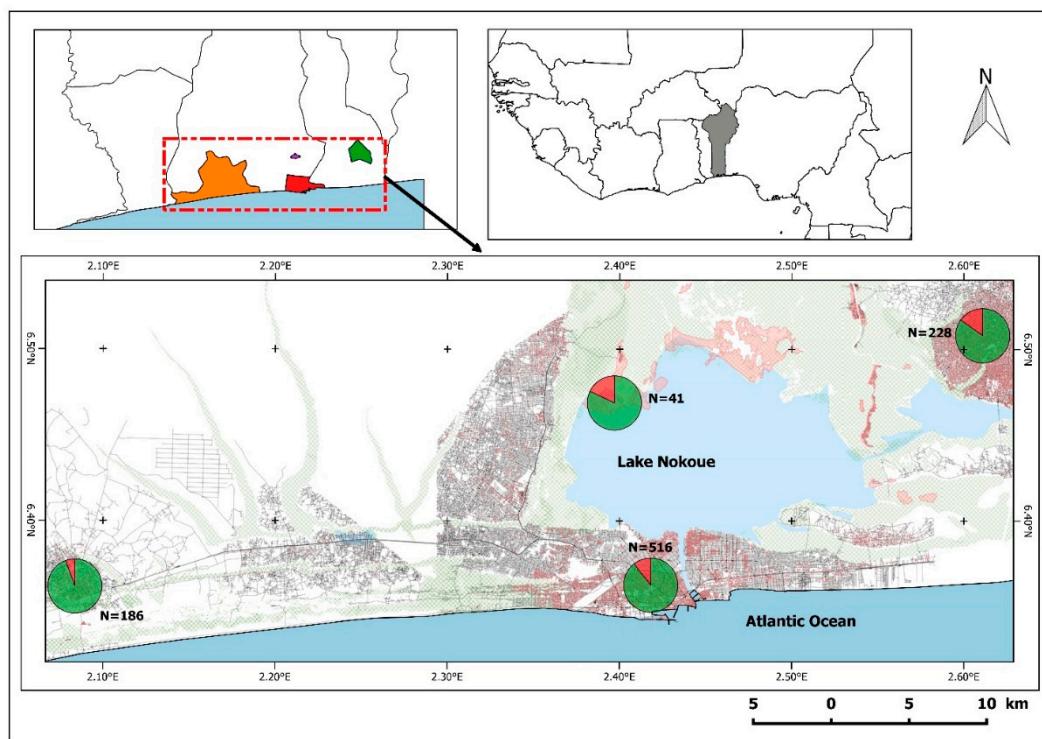
associated with water-related activities (e.g., animal breeding, sewer managing, fishing, rice culture, irrigated urban gardening, etc.), as well as heavy rainfall and flooding [2]. In such a context, urban leptospirosis became the focus of special attention (e.g., References [9,10]), since cities by essence gather high densities of both humans and commensal rodents. This is particularly true in tropical developing countries where urbanization may be extensive but uncontrolled, thus leading to the emergence of vast, very crowded and poorly sanitized areas where rats proliferate and where floods are recurrent, thus increasing the risk of leptospirosis transmission [11]. Yet, urban leptospirosis remains poorly documented in many countries, especially in Africa where data remain sparse [7,12]. For instance, the West African coastal corridor extends from Côte d'Ivoire to Nigeria and is expected to reach 34 million inhabitants by 2025, thus listing among the most populated conurbations in the world [13]. It already comprises large cities like Abidjan, Accra, Lomé, Cotonou, and Lagos, which shelter many very poor and informal settlements that develop within humid and highly floodable zones. Such conditions are supposed to be favorable for human leptospirosis, which is still widely overlooked in the region [14].

Southern Benin displays a subequatorial climate, with one short and one long dry season in August and from November to March, respectively. The long (April to July) and short (September and October) rainy seasons bring an average annual rainfall that reaches 1200 mm. Benin's Atlantic coastline is only 120 km long, and is characterized by an important mangrove fringe and its associated hydrographic network. Most of the coast is densely populated in an almost continuous urban conurbation that gathers Cotonou (1,561,000 inhabitants including Abomey-Calavi and Sémè-Kpodji), Ouidah (162,000 inhabitants), Porto-Novo (265,000 inhabitants), and their urban and peri-urban surroundings [15]. Recently, pathogenic leptospires were found in 18.9% of small mammals from Cotonou, Benin [16], thus demonstrating their circulation within the urban habitat. Serologic unpublished studies conducted in southern Benin, especially in Cotonou, also showed that leptospires could spread to people since 15% seroprevalences were found in randomly sampled inhabitants [17,18], reaching more than 50% and up to 76% in at-risk populations and febrile patients [17–19]. Unfortunately, the presence or absence of symptomatic cases of human leptospirosis remains undocumented in Benin, with one possible exception (Kpessou et al., submitted).

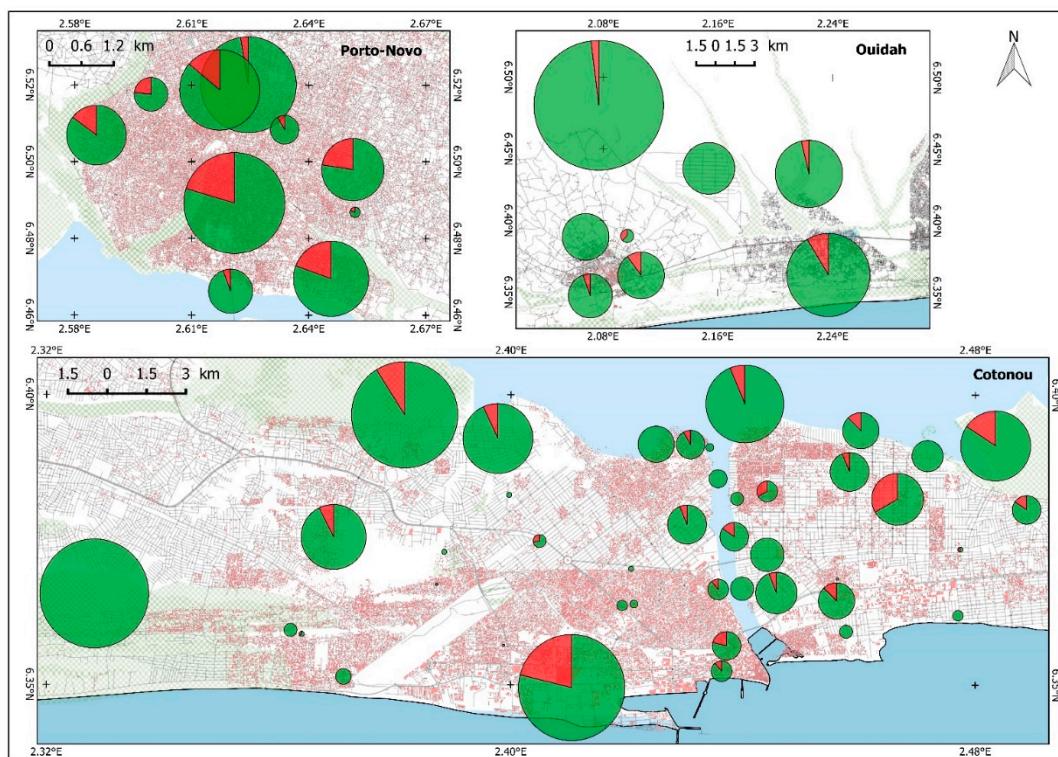
Here, we extend the study of *Leptospira* previously conducted on 90 small mammals from Cotonou [16] by monitoring 971 rodents and shrews from the three main cities (i.e., Cotonou, Porto-Novo, and Ouidah), as well as surrounding peri-urban localities of south Benin, in order to refine the role of commensal small mammals in the maintenance and circulation of pathogenic leptospires in the extensively urbanized West African coastal corridor.

## 2. Material and Methods

Sessions of small mammal capture were organized within four main zones that cover most of the urban corridor of southern Benin but that were investigated independently for logistical reasons (Figure 1; Figure 2; Table 1). Trapping within the Cotonou zone was conducted within the core city and its urban suburbs, as well as in Togbin, a mangrove village located around 4 km west of Cotonou, which should be part of its suburbs in the years to come following ongoing urbanization. Trapping sites within the Porto-Novo zone were all located within the town. Several localities were explored within the Ouidah area, which consists of urban as well as peri-urban to rural habitats. Finally, the fourth zone corresponded to the typical lacustrine village of Ganvié that lies on Lake Nokoué.



**Figure 1.** Sample size (green circles,  $N$ ) and overall rodent-borne *Leptospira* prevalence (red pie charts) in each of the four trapping zones from south Benin: Porto-Novo, Ouidah, Cotonou, and Ganvié, which correspond to the green, orange, red, and pink areas in the upper-left panel.



**Figure 2.** Sample size (green circles) and rodent-borne *Leptospira* prevalence (red pie charts) in the Porto-Novo, Ouidah, and Cotonou cities.

**Table 1.** Small mammal-borne pathogenic *Leptospira* prevalence at each zone and site in south Benin.

Zone	Locality/Site	Environment	GPS		All		Rra		Rno		Mna		Cro		Other	
			Lat	Long	N	Pos	N	Pos								
Cotonou	Cotonou/Abokicodji lagune	Urban	6.363	2.442	16	1	12	0			3	0	1	1		
	Cotonou/Adogléta	Urban	6.381	2.438	5	0	2	0	1	0			2	0		
	Cotonou/Agbato	Urban	6.390	2.439	8	4	5	1			3	3				
	Cotonou/Agontinkon	Urban	6.374	2.404	5	2	4	2					1	0		
	Cotonou/Ahouansorí	Urban	6.388	2.423	14	0	13	0	1	0						
	Cotonou/Aïbatin	Urban	6.358	2.363	5	0	5	0								
	Cotonou/Avotrou	Urban	6.389	2.476	27	5	19	2			7	3	1	0		
	Cotonou/Ayimlofidé	Urban	6.392	2.434	30	2	28	2	2	0						
	Cotonou/Bokossi Tokpa	Urban	6.365	2.438	9	0	2	0	5	0			2	0		
	Cotonou/Dandji	Urban	6.373	2.477	2	2	1	1			1	1				
	Cotonou/Dédokpo	Urban	6.369	2.441	13	0	7	0	4	0	2	0				
	Cotonou/Djidjé	Urban	6.384	2.434	7	0	3	0	2	0			2	0		
	Cotonou/Enagnon	Urban	6.362	2.453	14	2	9	1	2	1	1	0	2	0		
	Cotonou/Fidjrossé	Urban	6.350	2.370	6	0	4	0			2	0				
	Cotonou/Fifadji	Urban	6.395	2.398	41	4	39	3	2	1						
	Cotonou/Finagnon	Urban	6.361	2.476	4	0	4	0								
	Cotonou/Marché Ganhi	Urban	6.354	2.437	11	3			7	3			4	0		
	Cotonou/Gankpodo	Urban	6.393	2.456	15	1	12	0			1	1	2	0		
	Cotonou/Gbadji	Urban	6.372	2.388	2	0	2	0								
	Cotonou/Gbénonkpo	Urban	6.382	2.399	2	0					1	0	1	0		
	Cotonou/Godomey	Urban	6.413	2.312	13	0	12	0			1	0				
	Cotonou/Haie Vive	Urban	6.357	2.399	1	0	1	0								
	Cotonou/Houénoussou	Urban	6.358	2.364	2	1	1	1			1	0				

Table 1. Cont.

Zone	Locality/Site	Environment	GPS		All		Rra		Rno		Mna		Cro		Other	
			Lat	Long	N	Pos	N	Pos								
	Cotonou/Houéyaho	Urban	6.367	2.387	1	0	1	0								
	Cotonou/Kowégbô	Urban	6.387	2.469	12	0	11	0	1	0						
	Cotonou/Kpankpan	Urban	6.373	2.439	11	2			4	0	4	2	3	0		
	Cotonou/Ladji	Urban	6.389	2.433	11	1			7	1	3	0	1	0		
	Cotonou/Maher	Urban	6.392	2.434	3	0									3*	0
	Cotonou/Marché Dantokpa	Urban	6.374	2.430	15	1	12	1	2	1	1	0				
	Cotonou/Maro militaire	Urban	6.363	2.421	3	0	1	0	1	0	1	0				
	Cotonou/Minonchou	Urban	6.391	2.457	14	2	11	2	1	0	1	0	1	0	1	0
	Cotonou/PAC (harbour)	Urban	6.348	2.431	41	11	24	8	11	3			1	0	5**	0
	Cotonou/Sèdami	Urban	6.370	2.420	2	0	1	0	1	0						
	Cotonou/Sodjatinmin	Urban	6.368	2.456	1	0	1	0								
	Cotonou/Saint Jean	Urban	6.363	2.418	4	0	4	0								
	Cotonou/Saint Jacques	Urban	6.358	2.457	5	0	3	0			1	0	1	0		
	Cotonou/Suru Léré	Urban	6.382	2.462	20	10	19	9			1	1				
Cotonou	Cotonou/Tchankpamé	Urban	6.378	2.486	11	2	8	1			1	1	2	0		
	Togbin	Peri-urban	6.357	2.302	42	0	6	0			35	0	1	0		
	Cotonou/Tokpa Hoho	Urban	6.365	2.434	8	1	3	0	4	1			1	0		
	Cotonou/Vossa Kpodji	Urban	6.397	2.400	27	2	22	2	3	0	1	0	1	0		
	Cotonou/Wlacodji	Urban	6.351	2.442	8	1	3	0	2	1	1	0	2	0		
	Cotonou/Zogbohoué	Urban	6.379	2.389	25	2	23	2	1	0			1	0		
	Total				516	62	338	38	64	12	72	12	34	1	8	0
Ganvié	Ganvié	Lacustrial	6.468	2.397	41	9	35	6	5	3			1	0		
	Total				41	9	35	6	5	3			1	0		

Table 1. Cont.

Zone	Locality/Site	Environment	GPS		All		Rra		Rno		Mna		Cro		Other	
			Lat	Long	N	Pos	N	Pos								
Porto Novo	Porto-Novo/Adjinan	Urban	6.470	2.614	17	1	13	0							4	1
	Porto-Novo/Akonaboé	Urban	6.514	2.605	13	4	1	0	2	0	10	4				
	Porto-Novo/Djegan-Daho	Urban	6.487	2.651	24	7	4	1	1	1	9	2	10	3		
	Porto-Novo/Dowa	Urban	6.497	2.594	23	4	17	2			1	1	5	1		
	Porto-Novo/Gbékong	Urban	6.469	2.635	29	7	17	3			7	2	5	2		
	Porto-Novo/Grand Marché	Urban	6.475	2.630	39	10	12	8					27	2		
	Porto-Novo/Hounsa	Urban	6.511	2.634	11	1	2	1	1	0	2	0	6	0		
	Porto-Novo/Marché Ouando 1	Urban	6.505	2.612	37	1	32	1					5	0		
Ouidah	Porto-Novo/Marché Ouando 2	Urban	6.508	2.611	31	5	29	5					2	0		
	Porto-Novo/Zounkpa	Urban	6.484	2.649	4	1					1	1	3	0		
	Total				228	41	127	21	4	1	30	10	67	9		
	Savi/Minantinkpon	Peri-urban	6.383	2.091	5	3					3	2			2	1
	Savi/Houéton	Rural	6.428	2.104	20	0	10	0			8	0	2	0		
	Gakpé	Rural	6.435	2.111	50	1	41	0			7	1	2	0		
	Pahou/Marché de Pahou	Urban	6.384	2.208	32	3	14	0	6	3	2	0	10	0		
	Pahou/Adjarra	Peri-urban	6.409	2.200	26	1	19	0			6	1	1	0		
Total	Ouidah/Gbénan	Urban	6.372	2.068	18	0	17	0			1	0				
	Ouidah/Marché de Zobé	Urban	6.359	2.087	17	1	9	0			3	0	5	1		
	Ouidah/Marché de Kpassé	Urban	6.374	2.090	18	2					4	0	14	2		
Total					186	11	110	0	6	3	34	4	34	3	2	1
Total					971	123	610	65	79	19	136	26	136	13	10	1

Note: "Rra", "Rno", "Mna", and "Cro" stand for *Rattus rattus*, *R. norvegicus*, *Mastomys natalensis*, and *Crocidura cf. olivieri*, respectively. N and "pos" indicate the number of captured and of qPCR-positive individuals, respectively. "Lat" and "Long" represent latitude and longitude, respectively. \* *Dasymys rufulus*, \*\* *Mus musculus*, \*\*\* *Arvicantis* sp.

In each locality investigated, oral agreement was obtained from district heads. Work within private properties, either outdoors or indoors, was started only after our research purpose was explained, and a formal oral authorization was explicitly provided by the inhabitants.

Locally made wire-mesh traps or a combination of locally made wire-mesh and Sherman traps were used. Baits consisted of fish or a mixture of fish and peanut butter. Small mammals were trapped alive and brought to the lab where they were euthanized usually within the same day or, at maximum, within the next three days, using di-ethyl ether. A series of samples were performed for further genetic and epidemiologic analyses, including a piece of kidney that was preserved in 96% ethanol for the screening of *Leptospira*. Samples of each small mammal were data-matrixed and are now housed at the Center of Biology for Population Management (CBGP, France) collections [20], with the exception of one ethanol-preserved tissue sample that was systematically placed in the Abomey-Calavi University collections, Cotonou, Benin.

Molecular investigation of pathogenic leptospires followed previously described protocols [21]. In brief, individual genomic DNA was extracted from ethanol-preserved kidney tissue using the Biobasics 96-Well Plate Animal Genomic DNA Mini-Preps Kit. Whole DNA was eluted with 150  $\mu$ L of elution buffer and was quantified using Nanodrop technology (Thermoscientific). The presence of pathogenic *Leptospira* was scrutinized following a probe-based qPCR approach that targets a fragment of the *LipL32* gene, using a LightCycler® 480 (Roche Diagnostics) in 384-well microtiter plates with a 10- $\mu$ L final volume for each reaction. All host individuals were investigated in duplicate. When feasible, *Leptospira* species were identified in RT-PCR-positive small mammals through partial 16S gene sequencing.

Homogeneity of *Leptospira* species distribution between mammalian hosts was investigated on the basis of our own sequences together with the seven partial DNA sequences retrieved by Houéménou et al. (see Table 3 in Reference [16]) using a Fischer exact test under R Studio v3.5.0 [22].

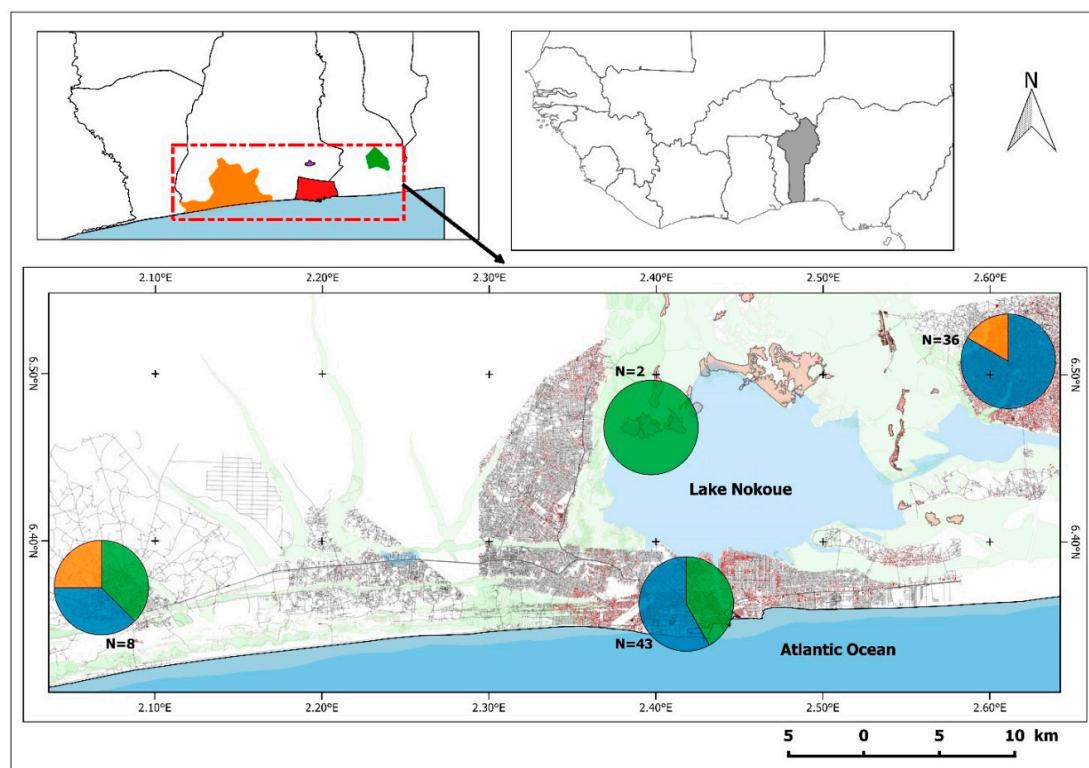
Trapping occurred between 2009 and 2017 (not shown); although such a pluri-annual sampling makes a proper seasonal survey difficult, months of capture were systematically noted in order to explore potential seasonal trends for prevalence. The Cotonou zone was mainly sampled in 2009 and 2010, with Fifadji being sampled in 2016, and Togbin and Ayimlofidé in 2017. The Porto-Novo, Ouidah, and Ganvié areas were investigated in 2015, 2015, and 2017, respectively. Seasonal variations of prevalence were explored using several datasets. Firstly, all data were pooled according to month of capture independently of the sites and the year of capture. Secondly, data from the Cotonou zone (i.e., Cotonou agglomeration and Togbin) were pooled by month independently of the year of capture (i.e., 2009–2010 for most of Cotonou sites, and 2017 for Togbin and Ayimlofidé). Thirdly, only data from the Cotonou agglomeration that covered the same yearly period (i.e., November 2009 to September 2010) were investigated. Lastly, we plotted monthly data from the Porto-Novo (September to November 2015) and Ouidah (August to December 2015) zones, respectively. In all instances, monthly prevalences were plotted together with monthly rainfalls, for which the 2009–2015 records were obtained from the Agency for Air Navigation in Africa and Madagascar (ASECNA) statistics service, Cotonou, Benin.

### 3. Results

In total, 971 small mammals were captured, including 610 *Rattus rattus*, 79 *R. norvegicus*, 136 *Mastomys natalensis*, two *Arvicanthis niloticus*, three *Dasymys rufulus*, five *Mus musculus*, and 136 shrews *Crocidura* cf. *olivieri*. Among them, 123 were found qPCR-positive for pathogenic leptospires, thus resulting in 12.7% overall prevalence (Table 1). Species-specific prevalences were variable between host species: they ranged from 9.6% in shrews to 10.7% in *R. rattus*, 19.1% in *M. natalensis*, and 24.1% *R. norvegicus*. Prevalence in rare species should be considered as poorly informative due to the very low sample sizes (50% in *A. niloticus* and 0% in both house mouse and *Dasymys rufulus*). Prevalences were also quite different between zones (i.e., 5.9%, 12%, 18%, and 22% in the Ouidah, Cotonou, Porto-Novo, and Ganvié zones, respectively; Table 1, Figures 1 and 2), as well as between sites (from 0% in several sites from the Cotonou and Ouidah areas to 60% in

Savi/Minantinkpon; Table 1 and Figure 2). Such important variations in prevalence were also observed more locally, i.e., between sites from the same zone: from 0% to 27.3% in Cotonou, 2.7% to 30.8% in Porto-Novo, and 0% to 60% in Ouidah (Table 1 and Figure 2).

A total of 89 *Leptospira* partial 16S sequences were retrieved. They belong to three phylogenetic lineages, namely, *L. borgpeterseni* ( $N = 58$ ), *L. interrogans* ( $N = 23$ ), and *L. kirschneri* ( $N = 8$ ). Distributions of these three species among zones, sites, and reservoir species are provided in Table 2. Although sample sizes did not allow us to perform proper statistical analyses, it can be noticed that *L. kirschneri*, *L. borgpeterseni*, and *L. interrogans* were all found in the Ouidah zone ( $N = 8$ ), while only *L. borgpeterseni* and *L. kirschneri* were detected in Porto-Novo ( $N = 36$ ), and only *L. interrogans* and *L. borgpeterseni* were retrieved in Cotonou ( $N = 43$ ). Only two sequences (both *L. interrogans*) were obtained from Ganvié (Figure 3). *L. interrogans* was found in all four reservoir species, while *L. borgpeterseni* was not identified in shrews. On the contrary, *L. kirschneri* was retrieved only in shrews. In nine instances, two *Leptospira* species were found to coexist within the same trapping site, sometimes in the same host species (Table 2). *Leptospira* species distribution (i.e., *L. kirschneri*, *L. borgpeterseni*, and *L. interrogans*) appeared highly significantly different between host species (i.e., *Crocidura cf. olivieri*, *Rattus rattus*, *R. norvegicus*, and *Mastomys natalensis*) (Monte Carlo (MC) simulated  $p$ -value on 2000 replicates =  $5 \times 10^{-4}$ ), with the highest residues pointing toward two significant preferential associations, namely, *L. kirschneri* in shrews and *L. interrogans* in *R. norvegicus*. The apparently strong association between *L. borgpeterseni* and *M. natalensis* (19 sequences out of the 20 retrieved in *M. natalensis* here and in Houéménou et al., 2013) did not appear as high, probably due to a similarly high association between *L. borgpeterseni* and *R. rattus* (37 out of 48 sequences from black rats). However, the residues of the *L. borgpeterseni/M. natalensis* association were much higher when black rats were removed from the analysis (data not shown), thus suggesting that south Benin multimammate rats shelter *L. borgpeterseni* more than expected under random conditions.



**Figure 3.** Relative frequencies of the various *Leptospira* species (as identified through 16S sequencing) in the four studied zones. Orange, blue, and green correspond to *L. kirschneri*, *L. borgpeterseni*, and *L. interrogans*, respectively.  $N$  indicates the number of sequences retrieved for each geographic zone (see also Table 2).

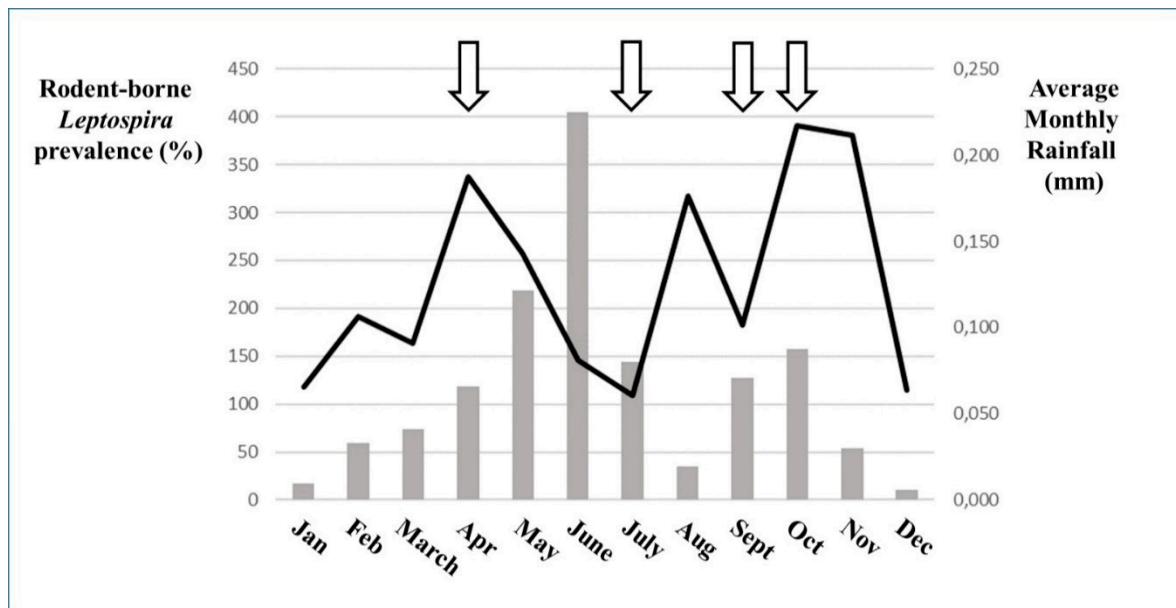
**Table 2.** *Leptospira* species (as identified by 16S sequencing) in the different host species, zones, and sites.

Zone	Site	Cro	Mna	Rno	Rra
Ouidah	Pahou Adjarra		1 int		
	Marché Zobé	1 kir			
	Marché Pahou			1 bor + 2 int	
	Minantinkpon		1 bor		
	Gakpé		1 bor		
Cotonou	Marché Kpassé	1 kir			
	Abokicodji	1 int			
	Agbato		2 bor		
	Agontikon				2 bor
	Avotrou		2 bor		1 bor
	Dandji		1 bor		
	Enagnon			1 int	1 int
	Fifadji			1 int	1 bor
	Ganghai			2 int	
	Gankpodo		1 bor		
Ganvié	Houenouussou				1 bor
	Kpankpan		2 bor		
	Marché Tokpa			1 int	
	PAC			1 bor + 1 int	6 int
	Suru Léré		1 bor		6 bor + 1 int
	Tchankpamé				1 bor
	Tokpa Hoho			1 int	
	Vossa Kpodji				1 bor + 1 int
	Wlacodji			1 int	
	Zogbohoué				2 bor
Porto-Novo	Ganvié				2 int
	Adjinan	1 kir			
	Ouando 1				1 bor
	Ouando 2				5 bor
	Grand Marché	1 kir + 1 bor			8 bor
	Djégan-Daho	3 kir	2 bor		2 bor
	Dowa	1 kir	1 bor		2 bor
	Akonaboé		2 bor		
	Gbékon	2 bor	2 bor		2 bor

Note: "Cro", "Mna", "Rno", and "Rra" stand for *Crocidura cf. olivieri*, *Mastomys natalensis*, *Rattus norvegicus*, and *Rattus rattus*, respectively. "kir", "bor", and "int" represent *L. kirschneri*, *L. borgpeterseni*, and *L. interrogans*, respectively.

Rodent-borne *Leptospira* could be detected in each season. Nevertheless, marked temporal variations in monthly prevalence were observed regardless of the dataset (all sites 2009–2017; Cotonou 2009–2017; Cotonou 2009–2010; Ouidah 2015; Porto-Novo 2015), ranging from 3.6% (Ouidah, September 2015) to 27.3% (Cotonou, November 2009). Interestingly, a trend showed prevalence peaks at the beginning (April and May) and end (October and November) of the rainy

season. Unexpectedly, low prevalence was retrieved in June and July when maximum rain falls. It was noted that prevalence peaks preferentially occurred during or one month after moderate rain (i.e., 100–200 mm). Such patterns are clearly illustrated in Figure 4, which takes into account all trapping sites and years.



**Figure 4.** Temporal variations of rodent-borne *Leptospira* prevalence (gray histograms) and average monthly rainfall (black line, calculated for the 2009–2015 period) as compiled from our whole dataset (four trapping zones, 2006–2016 period; see text for details). Arrows indicate average monthly rainfall between 100 and 200 mm.

#### 4. Discussion

Small mammals, especially rodents, constitute an important component of wild urban faunas and are implicated in the maintenance, circulation, and transmission (to humans) of a large range of zoonotic pathogens (reviewed in Reference [23]). Here, we observed that pathogenic *Leptospira* were present in rodents and shrews from most localities of south Benin. Using a 10-fold larger sample, we found a lower overall prevalence (12.7%) than the prevalence previously observed in 90 small mammals from Cotonou only (18.9% [16]), although the difference was not significant ( $\chi^2 = 2.7835$ , MC simulated  $p$ -value = 0.1). One plausible explanation for these slight variations could reside in sampling periods and places. Indeed, our main finding is that important fluctuations of prevalence exist in both space and time. For instance, deep differences in *Leptospira* prevalence in small mammals were observed between various areas of each city that were investigated at the same time. Our results also point toward important prevalence variations through time; peaks occurred at both the beginning (April and May) and the end (October and November) of the rainy/flooding seasons, with the highest prevalence values (>15%) being systematically retrieved during or one month after moderate monthly rainfall (i.e., 100–200 mm). This suggests that rodent-borne leptospires may be more prevalent when standing waters are present, but not at their highest levels (floods). This echoes what was retrieved in Madagascar, where higher prevalences were observed in animal reservoirs from the north of the country, which is drier than in the south of the island where rainfalls are abundant and the prevalence is lower [24]. If true, this would have important implications in terms of infection risk, as well as preventive actions, in the particular context of urban areas from coastal West Africa.

In south Benin, landscapes and water dynamics tightly interact to drive flood patterns. Standing waters are not randomly distributed, and they strongly depend on rainfall, water flow, and human-mediated shaping of the urban environment. This may also contribute to leptospirosis

risk being highly variable in both space and time in south Benin. In Brazilian slums, leptospirosis transmission to human was shown to be driven by very local processes such as rodent densities, proximity to dump sites, and lower altitude where waters converge, thus accounting for contamination hotspots [25,26]. Similar patterns were observed in Vancouver, Canada, where *Leptospira*-positive Norway rats were mostly grouped in given urban blocks [27]. Fine-scale and diachronic studies will be required in order to identify the determinants of a similarly heterogeneous spatio-temporal distribution of leptospires in the south Benin context.

Apart from the exceptional 80.3% obtained in Salvador City, Brazil [28], the small mammal-borne overall *Leptospira* prevalence observed in south Benin (18.9% in Reference [16]; 12.7% in the present study) is in good line with values from other urban settings (e.g., 10.5% in Vancouver, Canada [27]), including African ones (e.g., 14.5% in Durban, South Africa [29]; 18.3% in the Kibera slum of Nairobi, Kenya [30]). However, it is markedly higher than those observed so far in other West African cities (e.g., 1.5% in Conakry, Guinea [31]; 1.6% in Niamey, Niger [21]), including littoral ones (e.g., 4% in Abidjan, Côte d'Ivoire [32]). This suggests that leptospire circulation is particularly important in the urban environment of Cotonou and surrounding cities where rodent abundance is high (92% of infested houses; Dossou et al., unpublished). This is to be put in perspective with socio-environmental conditions (i.e., close interactions between reservoirs and people, extreme poverty, wide and long-standing flooding areas) that seem highly favorable for human contamination [14].

Animal reservoirs were sometimes found to be associated with particular *Leptospira* lineage (e.g., References [33,34]); however, to our knowledge, potential host specificity of *Leptospira* phylogenetic species remains poorly investigated. In Madagascar, the use of multi-locus genotyping analysis allowed Dietrich and colleagues [33] to demonstrate strong mammalian host specificity of endemic *Leptospira* lineages, as well as carriage of different *Leptospira* species by invasive animal reservoirs. They found that *L. borgpetersenii* and *L. kirschneri* were characteristic of endemic small mammals, while *L. interrogans* was observed only in introduced rats [33]. Here, we found similar trends of significantly preferential mammalian host/*Leptospira* species associations, with *L. kirschneri* more specifically found hosted by African shrews, while *L. interrogans* and *L. borgpeterseni* preferentially found associated with the invasive *R. norvegicus* and the native *M. natalensis*, respectively. At this stage, underlying processes for such an apparent—although imperfect—host/parasite specificity observed in southern Benin remain unknown. Invasion history by rats and associated leptospires may explain such a pattern. Alternatively, one may reasonably hypothesize that ecological conditions also intervene, with *R. norvegicus* preferring a more humid habitat where *L. interrogans* would be predominant, while *M. natalensis* may prefer slightly drier areas where *L. borgpetersenii* is more frequent. A similar type of habitat preference (i.e., *L. interrogans* in humid and floodable habitats, and *L. borgpetersenii* in both humid and dry but non-floodable habitats) was already noted in southeast Asia [35]. Unfortunately, whether or not such habitat preferences exist in urban small mammals from south Benin remains to be formally investigated (see References [36,37]). It should also be noted that *L. interrogans* was not identified in Porto-Novo in spite of the production of 36 sequences. Unfortunately, our data do not allow us to decipher between local ecological constraints that would not be favorable for this particular bacterial species and/or the absence of its main rodent reservoir, *R. norvegicus*, which was not sampled in this city.

Several studies showed that animal reservoirs other than rodents may be involved in *Leptospira* human infection [38,39]. For instance, domestic animals were proven to shelter pathogenic leptospires in neighboring countries, such as cattle in Nigeria (reviewed in Reference [12]) or dogs in Côte d'Ivoire [40]. This may also hold in Benin; dogs are relatively rare, especially in the most disadvantaged zones, but cats are sometimes used to get rid of rodents. Potentially more critical, divagating pigs, sheep, goats, and cattle are quite frequent. These animals circulate freely in cities or may sometimes be parked within highly populated and floodable areas. This situation could make these animals a potentially important source of pathogenic leptospires. Unfortunately, no data exist for leptospirosis in domestic species from Benin; thus, dedicated research is required.

The case of Ganvié is of special interest since it is a lacustrine village where houses are built on stilts pegged into Lake Nokoué and where moving requires a pirogue. Although an increasing number of inhabitants artificially created small islands through embankment, the permanence of free water greatly limits the presence of domestic animals. Only a few goats and hens can usually be kept on these artificial islands. It is, thus, highly probable that rodents are the main (and potentially only) reservoir for pathogenic leptospires in Ganvié. In such lacustrine peri-urban villages, rodents are abundant (Agossou et al., unpublished results), rodent-borne *Leptospira* prevalence is quite high (>20% in Ganvié), and water-related human activities are daily. As a consequence, people living in such an aquatic environment are expected to be at a particularly elevated leptospirosis risk.

To summarize, our study confirms and extends previous results on small mammal-borne *Leptospira* prevalence in south Benin cities, which was refined to 12%. However, very important variations seem to exist in both space and time, thus pointing toward the importance of local factors in leptospire distribution. In addition, possible trends in *Leptospira* host-specificity were observed, with *L. borgpetersenii*, *L. kirschneri*, and *L. interrogans* found preferentially in the native *Mastomys natalensis*, African shrew, and the invasive Norway rat, respectively. Whether this pattern is due to differences in host susceptibility, habitat preference by the hosts or the bacteria, and/or results from historical processes remains unknown. In any case, it is now clear that pathogenic leptospires are abundant in the environment of the Abidjan–Lagos corridor. Keeping in mind the socio-ecological conditions of this very rapidly urbanizing West African region, it is expected that local people are at high risk for leptospirosis. This is the reason why we recommend that epidemiological studies and awareness-raising campaigns be urgently conducted in the area.

**Author Contributions:** G.H. and G.D. conceptualized the study. G.H., J.E., S.B., H.-J.D., D.A., and G.D. conducted the field work. P.G. and M.P. performed the molecular analyses. G.D. wrote the first draft of the paper, which was amended by all authors.

**Funding:** This research was partly funded by the French Institute for Sustainable Development (IRD) and the French Pasteur Institute of Paris (IPP).

**Acknowledgments:** We are particularly grateful to all the people that allowed us to work inside and around their homes. Researches in the field were authorized following the agreement between the Republic of Benin and the Research Institute for Sustainable Development (IRD, France) (2009 and renewed in 2017), as well as between the University of Abomey-Calavi (Benin) and the IRD (30 September 2010).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Costa, F.; Hagan, J.E.; Calcagno, J.; Kane, M.; Torgerson, P.; Martinez-Silveira, M.S.; Stein, C.; Abela-Ridder, B.; Ko, A.I. Global morbidity and mortality of leptospirosis: A systematic review. *PLoS Trop. Neg. Dis.* **2015**, *9*, e0003898. [[CrossRef](#)] [[PubMed](#)]
2. Haake, D.A.; Levett, P.N. Leptospirosis in humans. *Curr. Top. Microbiol. Immunol.* **2015**, *387*, 65–97. [[PubMed](#)]
3. Bertherat, E.; Renaut, A.; Nabias, R.; Dubreuil, G.; Georges-Courbot, M.C. Leptospirosis and Ebola virus infection in five gold-panning villages in northeastern Gabon. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 610–615. [[CrossRef](#)] [[PubMed](#)]
4. Mattar, S.; Tique, V.; Miranda, J.; Montes, E.; Garzon, D. Undifferentiated tropical febrile illness in Cordoba, Colombia: Not everything is dengue. *J. Infect. Public Health* **2017**, *10*, 507–512. [[CrossRef](#)] [[PubMed](#)]
5. Santos, V.M.; Docha de Sa, D.A.; Turra, T.Z.; Ferreira Borges, N.M.; Nascimento, U.M.; Damasceno, E.A. Hantavirus pulmonary syndrome in Brasilia periphery: A diagnostic challenge. *J. Infect. Dev. Ctries* **2009**, *3*, 639–643. [[CrossRef](#)]
6. World Health Organization. *Investigation of Yellow Fever Epidemics in Africa; Field Guide*; WHO/HSE/EPR/2008.5; World Health Organization: Geneva, Switzerland, 2008.
7. Allan, K.J.; Biggs, H.M.; Halliday, J.E.B.; Kazwala, R.R.; Maro, V.P.; Cleaveland, S.; Crump, J.A. Epidemiology of leptospirosis in Africa: A systematic review of a neglected zoonosis and a paradigm for ‘One Health’ in Africa. *PLoS Trop. Neg. Dis.* **2015**, *9*, e0003899. [[CrossRef](#)] [[PubMed](#)]

8. Ellis, W.A. Animal leptospirosis. *Curr. Top. Microbiol. Immunol.* **2015**, *387*, 99–137. [PubMed]
9. Ko, A.I.; Reis, M.G.; Ribeiro Dourado, C.M.; Johnson, W.D.; Riley, L.W.; The Salvador Leptospirosis Study Group. Urban epidemic of severe leptospirosis in Brazil. *Lancet* **1999**, *354*, 820–825. [CrossRef]
10. Cornwall, W. A plague of rats. *Science* **2016**, *352*, 912–915. [CrossRef]
11. Lau, C.L.; Smythe, L.D.; Craig, S.B.; Weinstein, P. Climate change, flooding, urbanisation and leptospirosis: Fuelling the fire? *Trans. R. Soc. Trop. Med. Hyg.* **2010**, *104*, 631–638. [CrossRef] [PubMed]
12. De Vries, S.G.; Visser, B.J.; Nagel, I.M.; Goris, M.G.A.; Hartskeerl, R.A.; Grobush, M.P. Leptospirosis in Sub-Saharan Africa: A systematic review. *Int. J. Infect. Dis.* **2014**, *28*, 47–64. [CrossRef] [PubMed]
13. ONU Habitat. *L'état des Villes Africaines: Réinventer la Transition Urbaine*; ONU Habitat: Nairobi, Kenya, 2014.
14. Dobigny, G.; Gauthier, P.; Houéménou, G.; Choplin, A.; Dossou, H.J.; Badou, S.; Etougbéché, J.; Bourhy, P.; Koffi, S.; Durski, K.N.; et al. Leptospirosis and extensive urbanization in West Africa: A neglected and underestimated threat? *Urban Sci.* **2018**, *2*, 29. [CrossRef]
15. Direction des Études Démographiques. In *RGPH4: Que Retenir des Effectifs de la Population en 2013?* République du Bénin, Institut National de Statistique et de l’Analyse Économique: Cotonou, Benin, 2015.
16. Houemenou, G.; Ahmed, A.; Libois, R.; Hartskeerl, R.A. *Leptospira* spp. prevalence in small mammal populations in Cotonou, Benin. *ISRN Epidemiol.* **2013**. [CrossRef]
17. Bello, C.I. Prévalence et Facteurs Associés de la Séropositivité à la Leptospirose dans Certains Quartiers de Cotonou (Bénin). Ph.D. Thesis, Abomey-Calavi University, Cotonou, Benin, 2011.
18. Houéménou, G. Les Petits Mammifères de la Ville de Cotonou (Bénin): Peuvent-ils Constituer un Risque pour la Santé Humaine? Ph.D. Thesis, Liège University, Liège, Belgium, 2013.
19. Houngbo, P.T.; N’Gouizé, J. *Premiers Résultats de Dépistage Sérologique de la Leptospirose à Cotonou et Ses Environs*; DIT Report, TBH/ABM, Cotonou, Benin; CPU/UNB: Fredericton, NB, Canada; Saint John, NB, Canada, 1995.
20. Artige, E. *Collections Taxonomiques, Collections Vivantes et Ressources Génétiques pour la Biodiversité*; Technical and Research Document; Agropolis International: Montpellier, France, 2013; ISSN 1628-4240.
21. Dobigny, G.; Garba, M.; Tatard, C.; Loiseau, A.; Galan, M.; Kadaouré, I.; Rossi, J.P.; Picardeau, M.; Bertherat, E. Urban market gardening and rodent-borne pathogenic *Leptospira* in arid zones: A case study in Niamey, Niger. *PLoS Trop. Neg. Dis.* **2015**, *9*, e4097. [CrossRef] [PubMed]
22. R Project for Statistical Computing. 2018. Available online: <https://www.r-project.org/> (accessed on 1 April 2018).
23. Meerburg, B.; Singleton, G.R.; Kijlstra, H. Rodent-borne diseases and their risks for public health. *Crit. Rev. Microbiol.* **2009**, *35*, 221–270. [CrossRef] [PubMed]
24. Rahelinirina, S.; Leon, A.; Hartskeerl, R.A.; Sertour, N.; Ahmed, A.; Raharimanana, C.; Ferquel, E.; Garnier, M.; Chartier, L.; Duplantier, J.M.; et al. First isolation and direct evidence for the existence of large small mammal reservoirs of *Leptospira* sp. in Madagascar. *PLoS ONE* **2010**, *5*, e14111. [CrossRef] [PubMed]
25. Maciel, E.A.P.; de Carvalho, A.L.F.; Nascimento, S.F.; de Matos, R.B.; Gouveia, E.L.; Reis, M.G.; Ko, A.I. Household transmission of *Leptospira* infection in urban slum communities. *PLoS Trop. Neg. Dis.* **2008**, *2*, e154. [CrossRef] [PubMed]
26. Hagan, J.E.; Moraga, P.; Costa, F.; Capian, N.; Ribeiro, F.S.; Wunder, E.A.; Felzemburgh, R.D.M.; Reis, R.B.; Nery, N.; Sanata, F.S.; et al. Spatio-temporal determinants of urban leptospirosis transmission: Four-year prospective cohort study of slum residents in Brazil. *PLoS Trop. Neg. Dis.* **2016**, *10*, e0004275. [CrossRef]
27. Himsworth, C.G.; Bidulka, J.; Parsons, K.L.; Feng, A.Y.T.; Tang, P.; Jardine, C.M.; Kerr, T.; Mak, S.; Robinson, J.; Patrick, D.M. Ecology of *Leptospira interrogans* in Norway rats (*Rattus norvegicus*) in an inner-city neighborhood of Vancouver, Canada. *PLoS Trop. Neg. Dis.* **2013**, *7*, e2270. [CrossRef]
28. Tacunduva da Faria, M.; Calderwood, M.S.; Athanazio, D.A.; McBride, A.J.A.; Harstkerl, R.A.; Pereira, M.M.; Ko, A.I.; Reis, M.G. Carriage of *Leptospira interrogans* among domestic rats from an urban setting endemic for leptospirosis in Brazil. *Acta Trop.* **2008**, *108*, 1–5. [CrossRef]
29. Taylor, P.J.; Arntzen, L.; Hayter, M.; Iles, M.; Frean, J.; Belmain, S. Understanding and managing sanitary risks due to rodent zoonoses in an African city: Beyond the Boston model. *Integr. Zool.* **2008**, *3*, 38–50. [CrossRef] [PubMed]

30. Halliday, J.E.B.; Knobel, D.L.; Allan, K.J.; Bronsvoort, B.M.; Handel, I.; Agwanda, I.; Agwanda, B.; Cutler, S.J.; Olack, B.; Ahmed, A.; et al. Urban leptospirosis in Africa: A cross-sectional survey of *Leptospira* infection in rodents in the Kibera urban settlement, Nairobi, Kenya. *Am. J. Trop. Med. Hyg.* **2013**, *89*, 1095–1102. [[CrossRef](#)] [[PubMed](#)]
31. Zimmerman, S.; ter Meulen, A.; Fichet-Calvet, E.; Koivogui, L.; Sylla, O.; Goris, M.; Hartskeerl, R.A.; ter Meulen, J. Seroprevalence and reservoirs of leptospirosis in Conakry (Guinea). *Int. J. Med. Microbiol.* **2007**, *297*, 147–148. [[CrossRef](#)]
32. Bonfoh, B.; Mwachui, M.; Akpatou, B.; Zinsstag, J. Combined assessment of leptospirosis exposure in humans and rodents in Abidjan, Côte d'Ivoire. *Int. J. Infect. Dis.* **2014**, *21*, 220. [[CrossRef](#)]
33. Dietrich, M.; Wilkinson, D.A.; Soarimalala, V.; Goodman, S.M.; Dellagi, K.; Tortosa, P. Diversification of an emerging pathogen in a biodiversity hotspot: *Leptospira* in endemic small mammals of Madagascar. *Mol. Ecol.* **2014**, *23*, 2783–2796. [[CrossRef](#)] [[PubMed](#)]
34. Guernier, V.; Richard, V.; Nhan, T.; Rouault, E.; Tessier, A.; Musso, D. *Leptospira* diversity in animals and humans in Tahiti, French Polynesia. *PLoS Trop. Neg. Dis.* **2017**, *11*, e0005676. [[CrossRef](#)] [[PubMed](#)]
35. Cosson, J.F.; Picardeau, M.; Mielcarek, M.; Tatard, C.; Chaval, Y.; Suputtamongkol, Y.; Buchy, P.; Jittapalapong, S.; Herbreteau, V.; Morand, S. Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. *PLoS Trop. Neg. Dis.* **2014**, *8*, e2902. [[CrossRef](#)] [[PubMed](#)]
36. Houéménou, G.; Kassa, B.; Libois, R. Ecologie, diversité spécifique et abundance des petits mammifères de la ville de Cotonou au Bénin (Afrique de l'Ouest). *Int. J. Biol. Chem. Sci.* **2014**, *8*, 1202–1213. [[CrossRef](#)]
37. Dossou, H.J.; Houéménou, G.; Tenté, B.; Mensah, G.A. Inventaire des rongeurs et de leurs ectoparasites en milieu urbain au Bénin: Transmission possible de zoonoses. *Ann. Soc. Agron.* **2015**, *19*, 545–574.
38. Jobbins, S.E.; Alexander, K.A. Evidence of *Leptospira* sp. infection among a diversity of African wildlife species: Beyond the usual suspects. *Trans. R. Soc. Trop. Med. Hyg.* **2015**, *109*, 349–351. [[CrossRef](#)]
39. Guernier, V.; Lagadec, E.; Cordonin, C.; Le Minter, G.; Gomard, Y.; Pagès, F.; Jaffar-Bandjee, M.C.; Michault, A.; Tortosa, P.; Dellagi, K. Human leptospirosis on Reunion Island, Indian Ocean: Are rodents the (only) ones to blame? *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004733. [[CrossRef](#)] [[PubMed](#)]
40. Roqueplo, C.; Marié, J.L.; André-Fontaine, G.; Kodjo, A.; Davoust, B. Serological survey of canine leptospirosis in three countries of tropical Africa: Sudan, Gabon and Ivory Coast. *Comp. Immunol. Microbiol. Infect. Dis.* **2015**, *38*, 57–61. [[CrossRef](#)] [[PubMed](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).