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Research Article

Leptospira spp. Prevalence in Small Mammal Populations in Cotonou, Benin

G. Houemenou,[1] A. Ahmed,[2] R. Libois,[1] and R. A. Hartskeerl[2]

[1] *Unité de Recherche en Zoogéographie, Université de Liège, Bâtiment B22 Boulevard du Rectorat, 4031 Sart Tilman, Belgium*
[2] *WHO/FAO/OIE and National Leptospirosis Reference Centre, KIT Biomedical Research, Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands*

Correspondence should be addressed to G. Houemenou; houegual@yahoo.fr

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The aim of this study was to assess the *Leptospira* prevalence in small animals in Cotonou, the capital of Benin. Rodents and shrews were captured in urban and periurban settings and determined as species of the genera *Rattus, Mastomys,* and *Crocidura*. Kidney specimens of 90 animals were examined using a real-time PCR assay specific for leptospires that belong to pathogenic species. Leptospiral DNA was amplified from kidney tissues ranging from 13.3% (8/60) in *Rattus rattus* to 100.0% (1/1) in *Crocidura* spp. with an average of 18.9% (17/90) of the animals caught at 15 locations. Clade-specific Taqman PCR on 10 samples placed six of these within clade 1 comprising the species *L. kirschneri*, *L. interrogans, L. meyeri*, and *L. noguchii* and four within clade 2 consisting of species *L. weilii, L. alexanderi, L. borgpetersenii*, and *L. santarosai*. Phylogenetic analysis of partial sequences of the amplicons of seven samples of these 10 samples revealed that four of the clade 1 samples could equally be assigned to *L. interrogans* and *L. kirschneri* and three samples from clade 2 belonged to *L. borgpetersenii*. Results presented in the paper indicate that small mammals present a major public health risk for acquiring leptospirosis in Cotonou, Benin and will contribute to a raised awareness amongst health care workers and decision makers and hence promote appropriate clinical management of cases.

1. Introduction

Leptospirosis is one of the most common bacterial zoonoses worldwide [1]. In the past decade, leptospirosis has emerged as a globally important infectious disease. Although the disease has a worldwide distribution, it is particularly common in countries with humid tropical and subtropical climates [2].

Although rodents and insectivores are notorious reservoirs of leptospires, the vast majority of domestic and feral mammalian species can be natural hosts and transient carriers of *Leptospira* [3–7]. The serovar is the basic taxon of leptospires. To date, more than 250 serovars have been identified [4], each of these adapted to one or more hosts.

Mus musculus (house mouse), *Rattus norvegicus* (brown rat), and *Rattus rattus* (black rat) are three species of rodents with a worldwide distribution that are commonly associated with leptospiral infection [8].

Leptospires live in the kidneys and urogenital tract of the hosts and are excreted with the urine into the environment where they can survive for several months depending on favorable humid and warm conditions.

Humans are infected through direct contact with infected animals or, indirectly, by exposure to contaminated soil and water or through consumption of contaminated food or water [9]. Humans are dead-end hosts: the risk of human-to-human transmission probably is insignificant [1].

Leptospirosis infection in humans presents with symptoms that are similar to that of other better known parasitic, viral, and bacterial infections such as malaria, dengue, and other viral haemorrhagic fevers, salmonellosis, and brucellosis. Hence, leptospirosis is frequently misdiagnosed and, notably, its impact on African communities largely lacks documentation.

Although the disease is most common in tropical and rural settings, leptospirosis is considered as an increasing urban problem, mainly in rapidly growing cities in resource-limited regions where a lack of basic sanitation favors rodent-borne transmission [10].

Relatively few cases of leptospirosis are recorded on the African continent mainly due to unawareness and difficulties in diagnosis in both human and animals; hence the disease is not well investigated [11]. However, the prevailing climatic and socioeconomic environments are favorable for a high incidence of this disease [12].

The incidence of leptospirosis in West Africa is largely unknown and data on carrier rates in small mammals are scarce [13, 14]. In this region, leptospirosis has mainly been studied in Nigeria, a neighbor country of Benin, showing a high risk of exposure to leptospires. Application of the standard microscopic agglutination test (MAT) on sera collected from humans living in various parts of the country revealed a prevalence of 20.5% [15], while a serological survey in the Enugu area on distinct risk groups, including coal miners, butchers and slaughterhouse workers, farmers, and hospital laboratory personnel, revealed prevalence rates ranging from about 6 to 19% [16]. Serological and microbiological examination on slaughterhouse workers in the Nigerian state Plateau found a prevalence of 29.5% [17]. Furthermore, serological [18] and microbiological [19] investigations on potential natural hosts demonstrated infection rates ranging from 4.5% in brown field rats (*Arvicanthis niloticus*) to 23.5% in sheep.

In Benin, little is known about the actual impact of leptospirosis, particularly regarding the carrier rates in small mammals and the risk factors of human infection in urban zones. Previous investigations have been focused on human leptospirosis in Cotonou. A serological study on children hospitalized in the Centre National Hospitalier et Universitaire Hubert Koutoukou Maga (CHU) revealed a relatively low prevalence of 3.9% [22]. On the other hand, Koundé and Zohoun [23] reported a prevalence of about 20% in sera of apparently healthy blood donors in Centre National de Transfusion Sanguine de Cotonou and 66.5% in sera from slaughterhouse workers. Consistent with these findings, investigation on 503 serum samples from people working in slaughterhouses and 244 samples from febrile patients by MAT showed a positive outcome in 54.7% and 75.8%, respectively, of the samples [24].

These findings suggest that leptospirosis is an outstanding public health problem in Cotonou, Benin. However, the role that is played by rodents in urban epidemiology of this disease has remained unknown. The purpose of this study was to determine the prevalence of leptospirosis in small mammals in Cotonou as to assess their impact on urban leptospirosis.

2. Materials and Methods

The study was approved by the Ethical Committee of l'Université d'Abomey-Calavi of Benin, reference no. 208/MCOT/SG/DRH/DDCPRS/SFERM.

2.1. The Trapping Quarter Selection. According to resident population and the degree of sanitary infrastructure expressed in terms of potential risk of leptospirosis, we distinguished four types of areas in Cotonou town for trapping rodents encompassing various districts denoted as quarters: (A) areas at high risks of contracting leptospirosis: quarters on the shores of the lake Nokoué and the lagoon of Cotonou devoid of sanitary infrastructures; (B) areas at moderate risks: quarters with moderate sanitation; (C) areas with low risks: quarters consisting of areas with good sanitary infrastructures, including the embassy area; (D) nonresident putative high risk areas: market Dantokpa, market Ganhi, and Aïbatin (swamps).

2.2. Trappings. Trappings were done in 30 quarters in the risk areas as follows: 21 quarters in (A), 4 quarters in (B), 2 quarters in (C), and 3 in (D) (Figure 1(a)), and executed during November and December 2009 using specially made wire netting traps measuring $10 \times 10 \times 25$ cm.

Each trapping event was executed for three consecutive days (two nights) per quarter. Traps were baited with fish, set out in the morning of the first day, and checked for capturing early the second and third morning. Traps containing captured animals on the second morning were replaced. Captured rodents were collected and transported to the Laboratoire de Biologie Appliquée de l'Université d'Abomey-Calavi, Cotonou, Benin for determination.

2.3. Determination of Trapped Species. Trapped rodents were euthanized humanely by placing them in an iron box containing cotton wool soaked with chloroform or diethyl ether. Killed animals were then tagged for identification. Key criteria and illustrations developed by de Visser and coworkers [25] were used to identify the rodent species. Subsequently, animals were dissected aseptically and kidneys were obtained for molecular detection and identification of *Leptospira* and stored in alcohol (96% v/v) until DNA extraction.

2.4. Molecular Detection and Characterization of Leptospires. Analysis of kidney samples for the presence of leptospires was done on 90 specimens.

Approximately 25 mg of renal tissue was cut into small pieces and minced prior to DNA purification using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA extracts were diluted a 10-fold prior to amplification as to reduce the effects of residual inhibitors of the Taq polymerase, using the SYBR Green real-time PCR assay as previously described [3, 26].

For characterization, DNA was amplified and sequenced according to Victoria et al. [27]. Sequences of a panel of *Leptospira* spp. have been used as reference sequences to enable speciation. *Leptospira* strains and infections used in this study are listed in Table 1. DNA sequence alignments were generated with Vector NTI 10 software (Invitrogen). Multiple alignments of sequenced nucleotides were carried out using Clustal X (version 2) and the phylogenetic tree was drawn based on Neighbor-joining method [20] using MEGA5 software [21].

In addition, an adapted Taqman real-time PCR was applied that could separate *Leptospira* species in two clades of homologous species using clade-specific probes targeting *secY* gene, that is, one clade, denoted as clade 1, consisting

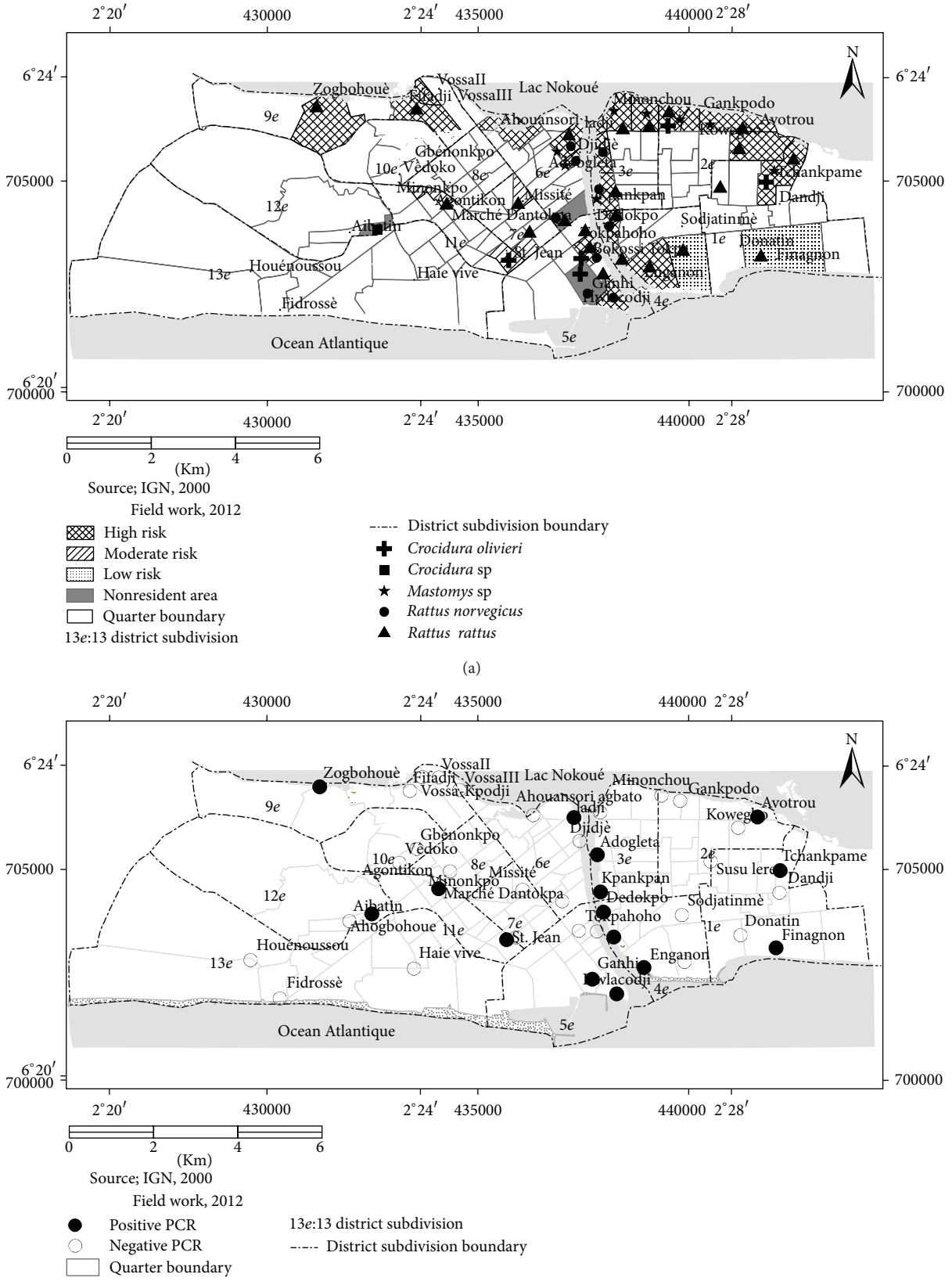

Figure 1: Map of Cotonou showing risk areas and catches of small mammals (a) and of *Leptospira* carriers based on positive PCR results (b). Symbols for risk areas and animal species are explained. *Mastomys* sp refers to unknown species of the genus *Mastomys* and *Crocidura* sp refers to unidentified species of *Crocidura*.

Table 1: *Leptospira* strains and infections used in *secY* sequence-based phylogeny.

Number	Code	Serovar	Strain	Species	Reference/genbank
1	1Bif.	Patoc	Patoc I	*L. biflexa*	NC_010842.1
2	2Bor.	Ballum	Mus 127	*L. borgpetersenii*	EU357953.1
3	3Bor.	Castellonis	Castellon 3	*L. borgpetersenii*	EU357955.1
4	4Bor.	Hardjo-bovis	JB197	*L. borgpetersenii*	CP000350.1
5	5Bor.	Tunis	P 2/65	*L. borgpetersenii*	EU358064.1
6	6Bor.	Nyanza	Kibos	*L. borgpetersenii*	EU358037.1
7	7Bor.	Tarassovi	Perepelitsin	L. borgpetersenii	EU358057.1
8	8Int.	Bratislava	Jez Bratislava	*L. interrogans*	EU357939.1
9	9Int.	Canicola	Hond Utrecht IV	*L. interrogans*	EU357961.1
10	10Int.	Gem	Simon	*L. interrogans*	EU358039.1
11	11Int.	Hardjo	Hardjoprajitno	*L. interrogans*	EU357983.1
12	12Int.	Hebdomadis	Hebdomadis	*L. interrogans*	EU357974.1
13	13Int.	Icterohaemorrhagiae	RGA	*L. interrogans*	EU357997.1
14	14Int.	Pomona	LT 1026	*L. interrogans*	EU358017.1
15	15Int.	Kuwait	136/2/2	*L. interrogans*	EU357970.1
16	16Int.	Lai	Lai	*L. interrogans*	AE010300.2
17	17Int.	Muenchen	München C 90	*L. interrogans*	EU357938.1
18	18Int.	Pomona	Pomona	*L. interrogans*	EU358013.1
19	19Kir.	Cynopteri	3522 C	*L. kirschneri*	EU358027.1
20	20Kir.	Kambale	Kambale	*L. kirschneri*	EU358030.1
21	21Kir.	Grippotyphosa	Moskva V	*L. kirschneri*	EU358028.1
22	22Kir.	Bim	1051	*L. kirschneri*	EU357952.1
23	23Kir.	Ramisi	Musa	*L. kirschneri*	EU358020.1
24	24Kir.	Mozdok	5621	*L. kirschneri*	EU358015.1
25	25Kir.	Ndambari	Ndambari	*L. kirschneri*	EU358001.1
26	26Kir.	Kamituga	Kamituga	*L. kirschneri*	EU357963.1
27	27Nog.	Argentiniensis	Peludo	*L. noguchii*	EU357960.1
28	28Nog.	Carimagua	9160	*L. noguchii*	EU358068.1
29	29Nog.	Huallaga	M 7	*L. noguchii*	EU357950.1
30	30San.	Beye	1537 U	*L. santarosai*	EU357981.1
31	31San.	Darien	637 K	*L. santarosai*	EU358066.1
32	32San.	Gatuni	1473 K	*L. santarosai*	EU358061.1
33	33San.	Rama	316	*L. santarosai*	EU358063.1
34	34San.	Rio	Rr 5	*L. santarosai*	EU358042.1
35	35San.	Shermani	1342 K	*L. santarosai*	EU357991.1
36	36San.	Tropica	CZ 299	*L. santarosai*	EU358005.1
37	37Wei.	Coxi	Cox	*L. weilii*	EU358009.1
38	38Wei.	Langati	M39039	*L. weilii*	EU358065.1
39	39Ben.	Undefined	418	*L. borgpetersenii*	This paper
40	40Ben.	Undefined	440	*L. interrogans*	This paper
41	41Ben.	Undefined	480	*L. interrogans*	This paper
42	42Ben.	Undefined	617	*L. kirschneri*	This paper
43	43Ben.	Undefined	823	*L. kirschneri*	This paper
44	44Ben.	Undefined	896	*L. borgpetersenii*	This paper
45	45Ben.	Undefined	202	*L. borgpetersenii*	This paper

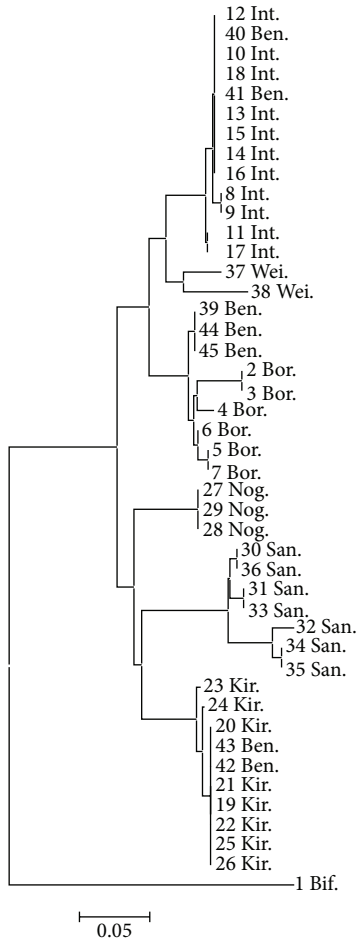

Figure 2: Phylogenetic tree of isolates from Cotonou and Leptospira reference strains. Phylogenetic tree of partial *secY* sequences. The evolutionary history was inferred using the neighbor-Joining method [20]. The optimal tree with the sum of branch length = 0.78179174 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). There were a total of 137 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 [21]. Codes of strains and infections are as listed in Table 1.

Table 2: Detection of leptospiral DNA by PCR in kidneys from rodents and shrews.

Specie	Number of animals	Number of positive PCR	Prevalence (%)
Crocidura olivieri	6	1	16.7
Crocidura spp.	1	1	100.0
Mastomys spp.	12	4	33.3
Rattus norvegicus	11	3	27.3
Rattus rattus	60	8	13.3
Total	90	17	18.9

of *L. kirschneri, L. interrogans*, *L. meyeri*, and *L. noguchii* and clade 2 comprising *L. weilii*, *L. alexanderi L. borgpetersenii,* and *L. santarosai*, identified by a 3′end FAM labeled probe and HEX labeled probe, respectively. The sequence of the primer pair and the probes used in this assay were as follows: primer Gte F, 5′-AA(T/C)GT(G/A)ATGCCGATCAT-3′, primer Gte R, 5′-GCGATTCAGTT(T/C)AA(C/T)CCTG-3; probe KIMN, 5′-AGTAGTCAAGAATGGGCTGGATG-FAM′3; probe WABS, 5′-AGTAGTCAAGAATGGGCT-GGATG-FAM′3.

In short, this TaqMan PCR was performed on a CFX96 Bio-rad real-time PCR (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA, USA) using LightCycler 480 Probes Master (Roche Applied Science, 68298 Mannheim, Germany). The following reaction conditions were used; reactions were performed in a total volume of 20 μL consisting of 1x LightCycler 480 Probes Master (Roche) of stock reagent containing FastStart Taq DNA polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP) and 6.4 mM $MgCl_2$. Forward and reverse primers were added at a final concentration of 600 nM each and the clade-specific probes at a final concentration of 150 nM each. The final concentration of target DNA was 250 pg/μL DNA. The amplification and hybridization protocol consisted of 5 min at 95°C, followed by 40 cycles of amplification and hybridization (95°C for 15 s, 60°C for 45 s). The resulting data were analyzed using the software provided by the CFX96 system (Bio-Rad CFX Manager version 2.0.)

3. Results and Discussion

3.1. Rodents Trapped according to Their Species. A total of 90 rodents and shrews were trapped and identified in order of decreasing number in Table 2 as *Rattus rattus*, *Mastomys* spp., *R. norvegicus*, *Crocidura olivieri*, and *Crocidura* spp. The majority of captures took place near fresh water sources, that is, the Aibatin swamp and the shores of Lac Nokoué and the river connecting the lake with the Atlantic Ocean, including the harbor area (Figure 1(a)). The capturing of a large number of *Rattus* spp. is consistent with their worldwide distribution [8] and the relatively high number of *Mastomys* spp. and *Crocidura* spp. is supported by previous studies in Africa [13].

In Cotonou, *R. rattus* was the most widely distributed rodent species in the study sample, originating from 24 of the 30 quarters, followed by *R. norvegicus* (9) and *Mastomys* spp. (8) (Figure 1(a)). This result is in agreement with the assumption that the black rat might be able to better adapt to climatologic and environmental conditions in West Africa, than the brown rat. Besides, the presence of *R. norvegicus* is generally restricted to coastal areas and sea ports while *R. rattus* has spread inland and is present in small towns and villages [28].

When separating rodent and insectivore species according to risk areas, we captured 53 *R. rattus,* eight *R. norvegicus,* twelve *Mastomys* spp., and three *Crocidura olivieri* in high risk areas (A); three *R. rattus* and one *Crocidura olivieri* in areas at moderate risk (B); two *R. rattus* in low risk areas (C); two *R. rattus*, three *R. norvegicus,* and three *Crocidura* spp., including two *Crocidura olivieri*, in putative high risk nonresident areas (D). The relatively high number of *Mastomys* spp. may

Table 3: *Leptospira* spp. associated with species of small mammals and trapping sites.

	Sample	Clade number by TaqMan*	Species according to phylogeny	Small mammal species	Trapping location**
1	202	*2*	*L. borgpetersenii*	*Mastomys* spp.	Tchankpamè
2	214	1	—	*Rattus norvegicus*	Ganhi +R
3	418	2	*L. borgpetersenii*	*Rattus rattus*	Agontinkon
4	440	1	*L. interrogans*	*Crocidura olivieri*	St Jean
5	476	1	—	*Rattus rattus*	Dédokpo R
6	480	1	*L. interrogans*	*Rattus norvegicus*	Adogléta R
7	617	1	*L. kirschneri*	*Crocidura* spp.	Aïbatin (Bas-fond)
8	823	1	*L. kirschneri*	*Rattus rattus*	Finagnon
9	896	*2*	*L. borgpetersenii*	*Rattus rattus*	Avotrou
10	1015	2	—	*Mastomys* spp.	Kpankpan R

*Clade 1 contains species *L. interrogans*, *L. kirschneri*, *L. noguchii*, and *L. meyeri*.
*Clade 2 contains species *L. borgpetersenii*, *L. santarosai*, *L. weilii*, and *L. alexanderi.*
**Trapping locations can be found in Figure 1.

be due to the presence of grasses around human dwellings near the lake (Figure 1(a)). This rodent may be found at a remote distance from habitations but is more frequently associated with markets, food stores, and farms [29].

3.2. Detection of Leptospiral DNA in Kidneys. We found a positive PCR signal in 17 of the 90 (18.9%) kidney samples with carrier rates ranging from 13.3 to 33% in different species, with the exception of the single infected animal identified as *Crocidura* spp. (Table 2). It should be noted that these percentages underestimate the actual prevalence. Kidney tissues are notorious for the presence of PCR-inhibitory substances in the extracted DNA samples [13, 26]. Although we have reduced the effect of inhibition by performing PCR on a 10-fold dilution, this may not exclude inhibition completely. Moreover, dilution also reduces concentrations of leptospiral target DNA below the lower limit of detection of the real-time PCR. Consequently, probably a proportion of infections have been missed and actual infection rates are higher than established. Unfortunately, it was not possible to assess the percentage of missed infections in this study.

All captured rodent and insectivore species had one or more PCR positive samples, suggesting that each of the species might be natural hosts for leptospires. These percentages are comparable to those in other reports from Africa. Investigations performed in South-eastern Africa, comprising Tanzania, Zimbabwe, Mozambique, and South Africa reported PCR positive results in about 11% of the rodent and insectivore populations, while culturing was successful in 1.6–7.4% of the animals [12, 13]. Moreover, high carrier rates in small mammals in this region of Africa were further substantiated by high seroprevalence rates, exceeding 40%, not only in small mammals but also in pet and farm animals as well as in humans (about 9–25%) [13]. Similar high seroprevalences have been reported in previous studies in Nigeria [18, 19], further substantiating that significant proportions of small mammals are infected with leptospires and present a severe hazard to the public health in the western region of Africa.

3.3. Geographic Distribution of Carriers. The 17 PCR-deduced infected small mammals were captured in 15 locations distributed in 50.0% (15/30) of the selected quarters. Distribution of infected populations of small mammals is associated with presumed levels of risk areas as based on the absence of sanitation or the presence of fresh water sources; of 15 quarters with proven carriers of leptospires, 10 belonged to presumptive high risk areas, including potential high risk nonresident quarters (Figure 1(b)). The nonresident areas (D) include the presence of markets and swamps that present excellent survival and proliferation conditions for small mammals. Therefore, we assume that these nonresident areas might be foci of animal reservoirs of leptospirosis. Our findings are consistent with previous reports stating that in urban areas, infection is associated with overcrowding, poor hygiene standards, inadequate sanitation, and poverty, all of which typically occur in urban slums in developing countries [30, 31].

3.4. Leptospira Species versus Species of Small Rodents. Determination of *Leptospira* species was achieved by two approaches. DNA samples that were positive in the SYBR Green real-time PCR were subsequently subjected to a TaqMan real-time PCR, enabling so separate *Leptospira* species into two clades consisting of *L. interrogans*, *L. kirschneri*, *L. noguchii*, and *L. meyeri* (denoted here as clade 1) and *L. borgpetersenii*, *L. santarosai*, *L. weilii*, and *L. alexanderi* (denoted as clade 2), respectively [27]. This already enables speciation at a reasonable level because most common species in Africa are *L. interrogans*, *L kirschneri*, and *L. borgptersenii*. The distribution of *L. santarosai* and *L. noguchii* is limited to the Americas and *L. weilli* is a species of southeast Asia [32], while other pathogenic species comprise low numbers of strains with an insignificant distribution or have a doubtful composition [3]. This implies that this approach allows the identification of *L. borgpetersenii* to a reliable level and the presence of *L. interrogans* and/or *L. kirschneri* can be determined as well, albeit that further discrimination between these two species is not possible.

Successful amplification by the Taqman PCR was obtained on 10 samples, separating 6 and 4 of these into clade 1 and clade 2, respectively (Table 3).

In seven cases a PCR product was obtained that allowed sequencing, of the product and subsequent phylogenetic analysis. Four of these PCR reactors were designated to clade 1; two could be identified as belonging to *L. interrogans* and two belonged to *L. kirschneri* suggesting that these two species are similarly present in Cotonou. As expected, all three samples that were initially placed into clade 2, were classified as *L. borgpetersenii* (Table 3).

PCR products, generated for sequencing, were relatively weak. A full sequence was only obtained from one sample (sample 418), while partial sequences were obtained from the other amplicons. To enable phylogenetic analysis all sequences were stripped to a segment of 139 bp comprising reliable sequence data for all products suitable for phylogenetic analysis (Table 1) [27]. The phylogenetic analysis revealed that the strains belonged to the species, *L. borgpetersenii*, *L. kirschneri*, and *L. interrogans* (Figure 2). Phylogenetic separation of *Leptospira* strains to an appreciable level on a 245 bp fragment of the *secY* gene has been reported before [26, 27] and is possible because of the high discriminative power of this gene [32–34]. However, the accuracy of this approach is hampered by further trimming the sequence down to about a half the size. Therefore, identification of the isolates on basis of this phylogenetic tree should be considered with some care. Nevertheless, our analysis shows that the *L. interrogans* isolates 440 and 480 are closely related to the serovars Gem, Hebdomadis, Icterohaemorrhagiae, Kuwait, Lai and Pomona. The *L. kirschneri* isolates 617 and 823 cluster together with serovars Cynopteri, Kambale, Grippotyphosa, Bim, Ndambari, and Kamituga. Sequences from isolates 418, 896, and 202 are identical and cluster with *L. borgpersenii* with serovar Kibos as one of the closest neighbours.

Detailed comparison with data from Africa is difficult due to the lack of survey related to the subject. Nevertheless, it should be noted that most of the related serovars within *L. interrogans*, *L. kirschneri*, and *L. borgpetersenii* originate or have been found in Africa or nearby islands (Gem, Kuwait, Kibos, Kambale, Ndambari, and Kamituga) and/or have a worldwide distribution (Icterohaemorrhagiae, Pomona, and Grippotyphosa) and, hence, support that the observations are consistent with the current knowledge on serovars circulating in this continent [35, 36].

Although the number of (presumptively) identified species is low, we looked for a pattern between the *Leptospira* spp. and the species of small mammals or location of trapping (Table 3). This allows some general conclusions. *L. borgpetersenii* seems to be confined to both *R. rattus* and *Mastomys* spp. On the other hand, *L. interrogans* and/or *L. kirschneri* was found in both *R. rattus* and *R. norvegicus* as well as in shrews (*Crocidura*). Presence of *L. interrogans* in shrews is not a common finding. Considering the short distances between the capture sites of rats and *Crocidura oliviera* in Cotonou (Figure 1(a)), it is conceivable to hypothesize that infection of *L. interrogans* in the shrews is maintained by transmission from the rat populations to the shrews. It is not clear from these data whether infections in the shrews are transient or whether the shrews form an infection reservoir of this usually highly virulent *L. interrogans* and hence present a persistent major risk for public health. Further research is needed to confirm or refute this possibility.

In conclusion, we have demonstrated that small mammals in Cotonou carry pathogenic leptospires at high rates. Thus our findings suggest a high exposure rate of human population in Cotonou to leptospirosis and predict leptospirosis as a high public health hazard. Further studies on potential infection reservoirs and on humans are needed to further substantiate this suspicion.

To date, health care workers and decision makers in Cotonou lack awareness of the presence of leptospirosis and diagnostic facilities are not in place. The current study as well as its continuation might raise alertness for this disease at public and veterinary health workers and decision makers in Benin. Because leptospirosis potentially is a treatable infectious disease, such increased awareness will contribute to better public and veterinary health care in Cotonou and in Benin in general.

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