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Spatio-temporal survey of small mammal-borne *Trypanosoma lewisi* in Cotonou, Benin, and the potential risk of human infection

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Highlights

- A qPCR- and 16SDNA sequencing-based search of *Trypanosoma* was conducted in 369 small mammals (i.e. 251 *Rattus rattus*, 18 *R. norvegicus*, 56 *Mastomys natalensis*, 2 *Praomys derooi*, 5 *Cricetomys* sp. and 37 *Crocidura* sp.) that were captured in three socio-environmentally degraded districts of Cotonou, Benin, during three different seasons of the same year.

- Sequencing data allowed us to unambiguously identify *Trypanosoma lewisi* in 144 parasitized animals.

- Prevalences were found to be very high with 57.2% of *Trypanosoma*-carrying individuals using qPCR only, and still up to 39.2% when considering only individuals for which 16S sequences were obtained.

- Although trypanosomes were unambiguously identified in all but one mammalian species investigated here, *Rattus rattus* was found to be significantly more infected than the other hosts. No difference was found neither between seasons nor districts.

- Taking into account the abundance of rodents within households, the very high rodent-borne *T. lewisi* prevalence as well as the tight promiscuity of inhabitants and rats in Cotonou, the risk of spill-over of trypanosomes from rodents to human appears very elevated in this type of urban habitat.

Abstract (300 words max)

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Human trypanosomoses are the sleeping sickness in Africa and Chagas disease in Latin America. However, atypical human infections by animal trypanosomes have been described, but poorly investigated. Among them, the supposed rat-specific *T. lewisi* was shown to be responsible for a few severe cases. In Africa, the scarcity of data and the null awareness about the atypical human trypanosomoses suggest that the number of cases may be higher than currently thought. Furthermore, *T. lewisi* is resistant to normal human serum and therefore a potential human pathogen. In order to document *T. lewisi* distribution and ecology, a qPCR- and 16S rDNA sequencing-based survey was conducted in 369 rodents from three urban districts of Cotonou city, Benin, during three different periods of the same year. Our study demonstrated very high prevalence (57.2%) even when considering only individuals identified as positive through DNA sequencing (39.2%). Black rats represented the most dominant as well as the most *T. lewisi*-parasitized species. No difference was retrieved neither between seasons nor districts, suggesting a large infestation of rodents by trypanosomes throughout the year and the city. Our results suggest that conditions are gathered for rat to human transmission of *T. lewisi* in these socio-environmentally degraded urban areas, thus pointing towards the rapidly urbanizing Abidjan-Lagos corridor as a region at particular risk.

Keywords: *Trypanosoma lewisi*, urbanization, environmental health, emerging disease, invasive species, rodent-borne zoonosis, Africa

INTRODUCTION

Since late 2000s, the World population has become more urban than rural for the first time in history (UN Population Division, 2014). Urbanization represents a drastic human-modified habitat, thus inducing deep environmental impacts (Grimm et al., 2008) that include shifts in infectious diseases' ecology (Alirol et al., 2011). As an example, cities are major transport hubs hence exchanges hotspots. As such, they are expected to be prone to biological invasions of highly adaptable species such as rats or mice. The latter species are reservoirs for a wide spectrum of zoonotic pathogens (review in Meerburg et al., 2009) that were disseminated worldwide through goods and people exchanges (e.g., plague: Schmid et al., 2015; hantavirus: Lin et al., 2012).

Trypanosoma lewisi and *T. lewisi*-like are trypanosomatid protozoans form a complex whose exact species-specific delineation within the subgenus *Herpetosoma* remains unclear (Hamilton et al.,

2007). They were mainly found in rodents, in Rattini in general, within the *Rattus* genus in particular (e.g., Linardi and Bothelo, 2002; Pinto et al., 2006; Dobigny et al., 2011; Milocco et al., 2011; Tang et al., 2012; Pumhom et al., 2014; Alias et al., 2014; Thompson et al., 2014; Ortiz et al., 2017; Tatard et al., 2017). It has sometimes been also identified in other rodent lineages (e.g., South American squirrel: Lainson et al., 2004; house mouse: Rodriguez et al., 2010; African spiny mouse: Dobigny et al., 2011; *Lophuromys*, *Hybomys*, *Lemniscomys*, *Praomys*, *Nannomys* and *Graphiurus* spp.: Salzer et al., 2016; *Mastomys* spp.: Ortiz et al., 2017; *Arvicanthis*, *Cricetomys*, *Mastomys*, *Nannomys* spp.: Tatard et al., 2017) as well as non-rodent species (e.g., shrews: Pumhom et al., 2014; marsupials: Pinto et al., 2006; bats: Fox and Thillet, 1962; primates: da Silva et al., 2010) including human (see below). In Africa, spill-over of *T. lewisi* from invasive rats to native rodent species has been suggested in at least three instances (Niger: Dobigny et al., 2011; Uganda : Salzer et al., 2016; Mozambique: Ortiz et al., 2017). It was usually considered as non-pathogenic for humans, although some human and sometimes transient and lethal infections have been documented in both Asia and Africa (e.g., Gambia: Howie et al., 2006; Thailand: Sarataphan et al., 2007; India: Kaur et al., 2007; Verma et al., 2011; reviewed in Truc et al., 2013). *T. lewisi* is now considered as potentially infective in humans since this parasite is resistant to normal human serum (NHS, Lun et al., 2015). However the extent of the risk for human health remains to be fully quantified (Brun, 2005). In addition, *in vitro* and *in vivo* experiments have shown that *T. lewisi* may have immunosuppressive effects in rats and reduce immune defense against co-infecting parasites such as *Toxoplasma gondii* or *Cryptococcus neoformans* (Arrea et al., 1998; Chinchilla et al., 2004, 2005; Gross et al., 2006). As a consequence, widescale circulation of *T. lewisi* among rodents may favour the proliferation of other environmental human pathogens. *T. lewisi* is transmitted by the contaminated feces of flea (Hoare, 1972).

T. lewisi is often associated to disturbed and/or highly anthropized habitats (Pumhom et al., 2014, 2015; Slazer et al., 2016). Indeed, we recently described high prevalences of *T. lewisi* in anthropophilic rodents from Niger and Nigeria villages and towns, with invasive rats, especially black rats probably playing an important role in their dissemination (Dobigny et al., 2011; Tatard et al., 2017). West African coastal harbour cities were previously suggested as a putative origin for invasive rats in this part of Africa (Berthier et al., 2016). This is the reason why we focus on the longitudinal study of small mammal-borne trypanosomes in three urban districts of Cotonou, Benin. We demonstrate very high prevalences all along the year in in all three study sites where black rats were abundant. The West African coastal urban corridor thus appears as an area where socio-environmental conditions seem highly favourable for *T. lewisi* spreading as well as to potential rodent-to-human transmission.

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MATERIAL and METHODS

Three districts of Cotonou, Benin, were monitored for rodent-borne trypanosomes: Agla (6,376N 2,362E), Ladji (6,389N 2,433E) and Saint-Jean (6,364N 2,416E) (Fig. 1). Agla is a relatively recent and poorly sanitized district that gets flooded at the beginning of the rainy season due to rainfall accumulation. Ladji is a very insalubrious, poor and densely inhabited area, located on the edge of Lake Nokoué that gets partly flooded at the end of the rainy season following increase of the lake level. Saint-Jean lies within the historic center of the city; it is a popular district where rainfalls may create large but short-term ponds. These three districts were selected in order to represent various socio-environmental conditions that can be found within Cotonou (Dansou, 2006; Sotindjo, 2009; PPCU3C, 2010).

Rodents' captures were conducted concomitantly in Agla (700 night-traps in total), Ladji (744 night-traps) and Saint-Jean (730 night-traps) at three different successive periods: at the end of the 2016 rainy season (ERS2016), during the 2017 dry season (DS2017) and at the beginning of the 2017 rainy season (BRS2017). In each district, the same 10 to 12 households were investigated at each period both inside (i.e. bedrooms, kitchens, store rooms, etc) and outside (i.e. courtyards) buildings. Locally made wire-mesh traps were used at the ERS2016 period, while Sherman (©Sherman) and locally made wire-mesh traps were used together at the DS2017 and BRS2017 periods. The use of these two types of traps was important since different species may be preferentially captured depending on the trap type (Garba et al., 2014). Baits was made of a mixture of peanut butter and fish. Traps were set for three consecutive nights and checked each morning. When a trap has captures, it was replaced by a new one, while empty traps were re-baited.

Rodents were brought alive at the lab where they were always euthanized using diethyl-ether and processed the same day. Unambiguous species-identification was performed on the basis of morphological features as well as genotyping and DNA sequencing (data not shown, but see Garba et al., 2014 for details). Only giant African rats and shrews could only be determined at the genus level (*Cricetomys* and *Crocidura*, respectively). Although the two latter taxa probably correspond to *C. emini* and *C. olivieri*, but in absence of relevant molecular data, we chose to refer them as to *Cricetomys* sp. and *Crocidura* sp., respectively. Fleas were collected following brushing immediately after sacrifice. Various samples were performed for genetic and epidemiologic investigations purpose, including an ethanol-preserved fragment of spleen that served for trypanosomes

investigation. Samples were deposited in the CBGP collections, Montpellier, France (Artige, 2013) as well as at the URIB, Cotonou, Benin.

Total DNA was extracted from rodent spleen samples and *Trypanosoma* presence was investigated using a qPCR procedure that was previously described (Dobigny et al., 2011; Tatard et al., 2017). All screening was performed in duplicates. A partial fragment of the *Trypanosoma* rDNA 16S gene was tentatively amplified and sequenced from qPCR-positive samples following published protocols (Dobigny et al., 2011; Tatard et al., 2017). Trypanosomes were then identified at the species-level using BlastN procedures as implemented in GenBank.

Statistics consisted in Khi2 tests and were performed under R Studio v1.1.447 (2018).

Explicit agreements of local traditional (e.g., family and household chiefs, shop, firm and garden owners) as well as administrative (i.e., Cotonou City Hall services, urban district chiefs) authorities were systematically obtained before trapping. None of the rodent species captured in the present study has protected status (see UICN and CITES lists). All animals were treated in a humane manner in accordance with guidelines of the American Society of Mammalogists (Sikes et al., 2011). Field works in Benin were conducted under the research agreement between Republic of Benin and the French Institute of Research for Development that was signed on 30th September 2010.

RESULTS

In total, 369 small mammals were investigated for *Trypanosoma* spp. Among them, 251 were *Rattus rattus*, 18 were *R. norvegicus*, 56 were *Mastomys natalensis*, 2 were *Praomys derooi*, 5 were *Cricetomys* sp. and 37 were shrews (*Crocidura* cf. *olivieri*). Samples among districts were distributed as follows: 158 in Agla (51, 47 and 60 in november 2016, march 2017 and june 2017, respectively), 109 in Ladji (36, 35 and 38) and 102 in Saint-Jean (36, 36, and 30). Species-specific distributions in both time and space can be deduced from Table 1.

In total, 154 sequences could be obtained: 144 of them strongly blasted to *T. lewisi*, while 5 retrieved no hit, 3 were poorly similar to rodent DNA (80-94% similarity) and 2 matched to another trypanosomatid genus, *Blechnomonas* (data not shown). Hereafter, only the 144 individuals from which sequences unambiguously similar to *T. lewisi* were considered as having provided a reliable 16S DNA sequence.

Prevalence were investigated using qPCR-positive individuals on the one hand, and qPCR-positive individuals from which unambiguous *T. lewisi* sequences could be retrieved on the other hand, thus providing upper and lower rodent-borne *T. lewisi* prevalence values, respectively.

When all qPCR-positive individuals were considered, overall small mammal-borne prevalence was 57.2% (211 individuals out of 369). When all three periods were pooled, local prevalences were 38% (60 out of 158), 61.5% (67 out of 109) and 82.4% (84 out of 102) in Agla, Ladji and Saint-Jean, respectively. Seasonal prevalence over all three districts reached 56.9% (70 out of 123), 66.9% (79 out of 118) and 48.4% (62 out of 128) in november 2016, march 2017 and june 2017, respectively. When focusing only on qPCR-positive individuals for which a *T. lewisi* sequence could be obtained, these prevalences remain very high (39.2%), ranging from 31% to 47.7% by districts and from 28.9% to 53.4% by season (see Table 1).

Within each district, no difference prevalence was found between seasons neither with qPCR (all $p > 0.29$) nor sequences (all $p > 0.1$) data. For each season, no difference could be retrieved between district (all $p > 0.24$ for both qPCR and sequence-based prevalence data), except BRS2017 which displayed a marginally significant difference in qPCR-based prevalence ($p = 0.044$). This difference was not significant when looking at sequence-based values ($p = 0.245$). As a consequence, we considered that no difference in overall prevalence existed nor between seasons nor between districts.

When focusing on the four well sampled species (i.e. 251 *Rattus rattus*, 18 *R. norvegicus*, 56 *Mastomys natalensis* and 36 *Crocidura cf. olivieri*), host species-specific prevalences were found significantly different, ranging from 33.3% in Norway rats to 66.9% in black rats with qPCR-based prevalence ($X^2 = 360.12$, $df = 3$, $p < 2.2 \times 10^{-16}$), and from 11.1% in shrews to 49.8% in black rats with sequence-based prevalence ($X^2 = 301.32$, $df = 3$, $p < 2.2 \times 10^{-16}$). Both qPCR- and sequence-based species-specific prevalences by season and by district are detailed in Table 1. Note that, within the two rarer species, one *Praomys derooi* (out of two) and three *Cricetomys* sp. (out of five) were found positive using qPCR, while two 16S *T. lewisi* sequences could be retrieved from two cricetomes (data not shown).

The number of sites where small mammals could be captured and *Trypanosoma* qPCR-positive ones were found were quite high: 7 sites out of 10, 9 sites out of 10 and 4 sites out of 10 in Agla at ERS2016, DS2017 and BRS2017, respectively; 7 sites out of 10, 7 sites out of 10 and 9 sites out of 9 in Ladji at each season respectively; 7 sites out of 9, 8 sites out of 9 and 8 sites out of 8 in Saint Jean at each season, respectively (see Fig. 2-4). Overall, this means that 73.3% of the trapping sites where small mammals were captured have harbored *Trypanosoma* qPCR-positive small mammals at least

once. Out of the 34 trapping sites that were investigated at least once during this study, only five never provided any qPCR-positive animals. However, the number of captures in these sites were usually very low (one site with 2 captures in Saint Jean; two sites with 8 and 2 captures in Agla; two sites with 1 and 2 captures in Ladji; data not shown).

Site-specific prevalences range from 0% to 100% whatever the technique used (i.e., qPCR or sequence; Fig. 2-4 and Suppl. Fig. 1-3). When one considers only the sites where at least 10 rodents could be investigated, prevalences range from 25% to 93% with qPCR, and from 16% to 60% with sequence (data not shown, but see Fig. 2-4 and Suppl. Fig. 1-3).

Discussion

This survey of small mammal-borne *T. lewisi* in Cotonou, Benin, clearly shows that this parasite is very widespread in all investigated urban areas (overall prevalence of 39.2% to 57.2% using only qPCR and confirmed sequences, respectively). In addition, we found that these high prevalence did not display noticeable seasonality. Taking into account the abundance of commensal small mammals together with the infestation level of households in Cotonou, this means that *T. lewisi* circulates extensively all over the year in the urban environment of southern Benin. The black rat, which is one of the most important reservoir species of *T. lewisi* (Tatard et al., 2017) as well as the most widespread commensal small mammal in Southern cities of Benin (Houéménou et al., 2013, 2019; Dossou et al., 2015), showed extremely high prevalences (49.8% with confirmed sequences, and up to 66.9% with qPCR only) that were significantly higher than those observed in the other host species. As a comparison, rodent-borne prevalences reach 13.9% in Mali (53.8% in black rats; Schwan et al., 2016), 14.4% in Uganda (29.5% in black rats; Salzer et al., 2016), 14.6% in Niger and Nigeria (25.2% in black rats; Tatard et al., 2017), 15.5% in Senegal (27.8% of black rats; Cassan et al., 2018) and up to 55.1% in Mozambique (40% of five black rats; Ortiz et al., 2017). Although spatial scales and methods used may potentially impact these results, thus limiting a rigorous quantitative comparison, it clearly appears that Cotonou is a hotspot for rodent-borne *T. lewisi* in Africa. By extension, this probably holds true for the whole extensively urbanizing Abidjan-Lagos corridor that shelters one of the largest African conurbation (UN Habitat, 2014).

In South East Asia, Pumhom and colleagues (2015) found that *T. lewisi* was more prevalent in rodents from highly anthropized habitats, with a strong association to built-up areas. They speculate that

such high prevalences may be characteristic of unstable hence expanding populations of rodent hosts, and that stable adult rodent populations may never show high prevalence unless the parasite was recently introduced (Pumhom et al., 2015). Here, we focused only an urban environment; we thus cannot rigorously compare prevalences in anthropized vs. non-anthropized habitats.

Nevertheless, we clearly observed very high prevalences, much higher than those reported in Asia (e.g., 9.8% on average, and up to 48.8% in the most anthropized areas such as Bangkok; Pumhom et al., 2014), thus confirming a strong association between urban rat populations and wide circulation of *T. lewisi*. However, we do not believe that black rat (the main trypanosome host identified here) populations from Cotonou are currently unstable and particularly expanding. Although the city is relatively recent (end of the 19th / beginning of the 20th centuries; Ciavolletta & Choplin, 2018), black rats are abundant all over the cities of southern Benin, including Cotonou and the urban surrounding areas (Dossou et al., 2015 ; Houéménou et al., 2013, 2019). Moreover, our unpublished population genetics data show that they form one single functional and well-diversified population at the scale of the whole city (Badou et al., unpublished data).

Our results confirm that *T. lewisi* shows significant host species specificity, with black rats displaying from far the highest prevalence. Interestingly, however, *T. lewisi* was also unambiguously identified (i.e., a parasite sequence was obtained) on some native hosts (9 and 2 *T. lewisi* sequences obtained from *Mastomys natalensis* and *Cricetomys* sp., respectively), including native shrews (4 sequences). This agrees with previous studies which demonstrate that *T. lewisi* is able to infect a wide range of rodents (reviewed in Ortiz et al., 2017) as well as non-rodent mammalian hosts (e.g., Primates, Eulipotyphla, Lagomorpha, Macroscelidea, Chiroptera; Hoare et al., 1972; da Silva et al., 2010). The spill-over from rats to other rodents or non-rodent mammals was suggested by several authors, including in Africa (Dobigny et al., 2011; Salzer et al., 2016; Tatard et al., 2017; Ortiz et al., 2017). Although rats, especially black rats, were suggested to play a major role in trypanosome circulation in West Africa, especially in Senegal (Cassan et al., 2018), Niger and Nigeria (Dobigny et al., 2011; Tatard et al., 2017), we here sometimes found quite important prevalence on non-*Rattus*, native hosts (e.g., 33.9% and 16.1% in *M. natalensis* with qPCR and sequence, respectively). If one considers that black rats are currently invading this part of Africa from coastal cities of the Guinea Gulf towards the hinterland (Berthier et al., 2016; Hima et al., in prep.), one may speculate that *Trypanosoma* spill-over from rats to native rodents all along a latitudinal gradient of bioinvasion. In other words, invasive rats and native rodents would have coexisted for a longer time in southern localities (like Cotonou), thus leading to a more effective spill-over and resulting of higher prevalence. On the contrary, rats and native rodent species would have experienced a shorter period of contact (like in

Niamey; Berthier et al., 2016), thus leading to more restricted spill-over and lower prevalence of trypanosomes. Note that such an invasive-to-native spill-over of *T. lewisi* has already been found to parallel the successful bioinvasion of black rats at the expense of the endemic *Rattus macleari* which disappeared from Christmas Island (Norris & Pickering, 1996; Wyatt et al., 2008).

Trypanosomes spill-over is also reflected by human cases of *T. lewisi* symptomatic infection (reviewed in Truc et al., 2013). Such rat-to-human spill-overs of atypical trypanosomes may be rare or, alternatively, may be largely overlooked (da Silva et al., 2010; Truc et al., 2013). If this was to be the case, one may expect West African urban dwellers to be at high risk of trypanosome contamination. Indeed, the strong abundance of rats together with the high number of rat-infested households, as well as the very high rodent-borne prevalence observed in Cotonou constitute three important risk factors. In addition, although rigorous flea sampling protocols were not applied, fleas were found in 19% of the small mammals included in the present study (data not shown); this means that the vector is also widely distributed. Furthermore, rats are living within households and their nests are usually dig inside homes, sometimes only a few centimeters away from beds or mats. Young children, who may be among the most vulnerable to *T. lewisi* infections (Truc et al., 2013), are often let asleep alone in these bedrooms. This puts them at high risk of being infested by fleas that have abandoned rats dead in their nests. Such events may be quite frequent in Cotonou where people often use street-sold poisons to fight pest rodents (39.7% of 141 interviewed persons in Cotonou; Odou et al., unpublished data). Furthermore, young children could be infected by drinking fruit juice contaminated by flea feces. In other words, all conditions seem gathered for *T. lewisi* transmission to humans in poor areas of Cotonou city. Once again, since *T. lewisi* was morphologically identified in rodents from Côte d'Ivoire, Ghana and Nigeria (Hoare, 1972), there is little doubt that many currently urbanizing areas of the Abidjan-Lagos corridor may display similar risks. This is the reason why we believe this region would be a very pertinent study site to investigate the real burden of atypical trypanosomiasis in humans.

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Table and figure legends

Table 1: Results of small-mammal trapping and small-mammal borne *Trypanosoma lewisi* screening in Agla, Ladjé and Saint-Jean districts, during three successive periods (i.e. ERS2016: end of the rainy season 2016; DS2017: dry season 2017; BRS2017: beginning of the rainy season 2017). Overall as well as some species-specific results are provided. "N" indicates the total number of investigated individuals, while "qPCR", "seq" and "%" stand for qPCR-based, sequence-based number of positive individuals as well as corresponding prevalence, respectively. Data for rarer species (namely *Praomys derooi* and *Cricetomys* sp.) were not included (refer to text for details).

Figure 1: Map of Cotonou city, Benin, showing the location of the three districts investigated in the present study.

Figure 2: Maps of trapping sites investigated in Agla district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of

each circle is proportional to the number of captures at a given site, while pie charts indicate the number of qPCR-identified positive animals.

Figure 3: Maps of trapping sites investigated in Ladji district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of each circle is proportional to the number of captures at a given site, while pie charts indicate the number of qPCR-identified positive animals.

Figure 4: Maps of trapping sites investigated in Saint-Jean district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of each circle is proportional to the number of captures at a given site, while pie charts indicate the number of qPCR-identified positive animals.

Supplementary Figure 1: Maps of trapping sites investigated in Agla district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of each circle is proportional to the number of captures at a given site, while pie charts indicate the number of positive animals as identified by both qPCR and sequence data.

Supplementary Figure 2: Maps of trapping sites investigated in Ladji district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of each circle is proportional to the number of captures at a given site, while pie charts indicate the number of positive animals as identified by both qPCR and sequence data.

Supplementary Figure 3: Maps of trapping sites investigated in Saint-Jean district at the end of the rainy season 2016 (ERS2016), the dry season 2017 (DS2017) and beginning of the rainy season 2017 (BRS2017). Size of each circle is proportional to the number of captures at a given site, while pie charts indicate the number of positive animals as identified by both qPCR and sequence data.

Table 1: Results of small-mammal trapping and small-mammal borne *Trypanosoma lewisi* screening in Agla, Ladji and Saint-Jean districts, during three successive periods (i.e. ERS2016: end of the rainy season 2016; DS2017: dry season 2017; BRS2017: beginning of the rainy season 2017). Overall as well as some species-specific results are provided. “N” indicates the total number of investigated individuals, while “qPCR”, “seq” and “%” stand for qPCR-based, sequence-based number of positive individuals as well as corresponding prevalence, respectively. Data for rarer species (namely *Praomys derooi* and *Cricetomys* sp.) were not included (refer to text for details).

District	Period	All small mammals					<i>Rattus rattus</i>					<i>Rattus norvegicus</i>					<i>Mastomys n.</i>		
		N	qPCR	%	seq	%	N	qPCR	%	seq	%	N	qPCR	%	seq	%	N	qPCR	%
Agla	ERS2016	51	20	39.2	15	29.4	37	15	41	13	35	3	2	66.6	2	66.6	10	3	33.
	DS2017	47	27	57.4	23	48.9	29	20	69	18	62.1	1	0	0	-	-	13	6	46.
	BRS2017	60	13	21.7	11	18.3	20	10	50	9	45	10	1	10	1	10	19	1	5.3
	all seasons	158	60	38	49	31	86	45	0.52	40	46.5	14	3	21.4	3	21.4	42	10	23.
Ladji	ERS2016	36	22	61.1	15	41.7	31	20	64.5	14	45.2	1	1	100	0	0	1	0	0
	DS2017	35	25	71.4	20	57.1	34	24	70.6	20	58.8	1	1	100	0	0	-	-	-
	BRS2017	38	20	52.6	17	44.7	27	16	59.3	14	51.9	2	1	50	1	50	1	1	100
	all seasons	109	67	61.5	52	47.7	92	60		48		4	3	75	1	25	2	1	50
Saint-Jean	ERS2016	36	28	77.8	14	38.9	32	26	81.3	14	43.8	0	-	-	-	-	1	1	100
	DS2017	36	27	75	20	55.6	22	19	86.4	16	72.7	0	-	-	-	-	9	5	55.
	BRS2017	30	29	96.7	9	30	19	18	94.7	7	36.8	0	-	-	-	-	2	2	100
	all seasons	102	84	82.4	43	42.2	73	63	86.3	37	50.7	0	-	-	-	-	12	8	66.
All districts	ERS2016	123	70	56.9	44	35.8	100	61	61	41	41	4	3	75	2	50	12	4	33.
	DS2017	118	79	66.9	63	53.4	85	63	74.1	54	63.5	2	1	50	0	0	22	11	50
	BRS2017	128	62	48.4	37	28.9	66	44	33.3	30	45.5	12	2	16.7	2	16.7	22	4	18.
	all seasons	369	211	57.2	144	39.2	251	168	66.9	125	49.8	18	6	33.3	4	22.2	56	19	33.

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Highlights

- A qPCR- and 16SDNA sequencing-based search of *Trypanosoma* was conducted in 369 small mammals (i.e. 251 *Rattus rattus*, 18 *R. norvegicus*, 56 *Mastomys natalensis*, 2 *Praomys derooi*, 5 *Cricetomys* sp. and 37 *Crocidura* sp.) that were captured in three socio-environmentally degraded districts of Cotonou, Benin, during three different seasons of the same year.
- Sequencing data allowed us to unambiguously identify *Trypanosoma lewisi* in 144 parasitized animals.
- Prevalences were found to be very high with 57.2% of *Trypanosoma*-carrying individuals using qPCR only, and still up to 39.2% when considering only individuals for which 16S sequences were obtained.
- Although trypanosomes were unambiguously identified in all but one mammalian species investigated here, *Rattus rattus* was found to be significantly more infected than the other hosts. No difference was found neither between seasons nor districts.
- Taking into account the abundance of rodents within households, the very high rodent-borne *T. lewisi* prevalence as well as the tight promiscuity of inhabitants and rats in Cotonou, the risk of spill-over of trypanosomes from rodents to human appears very elevated in this type of urban habitat.

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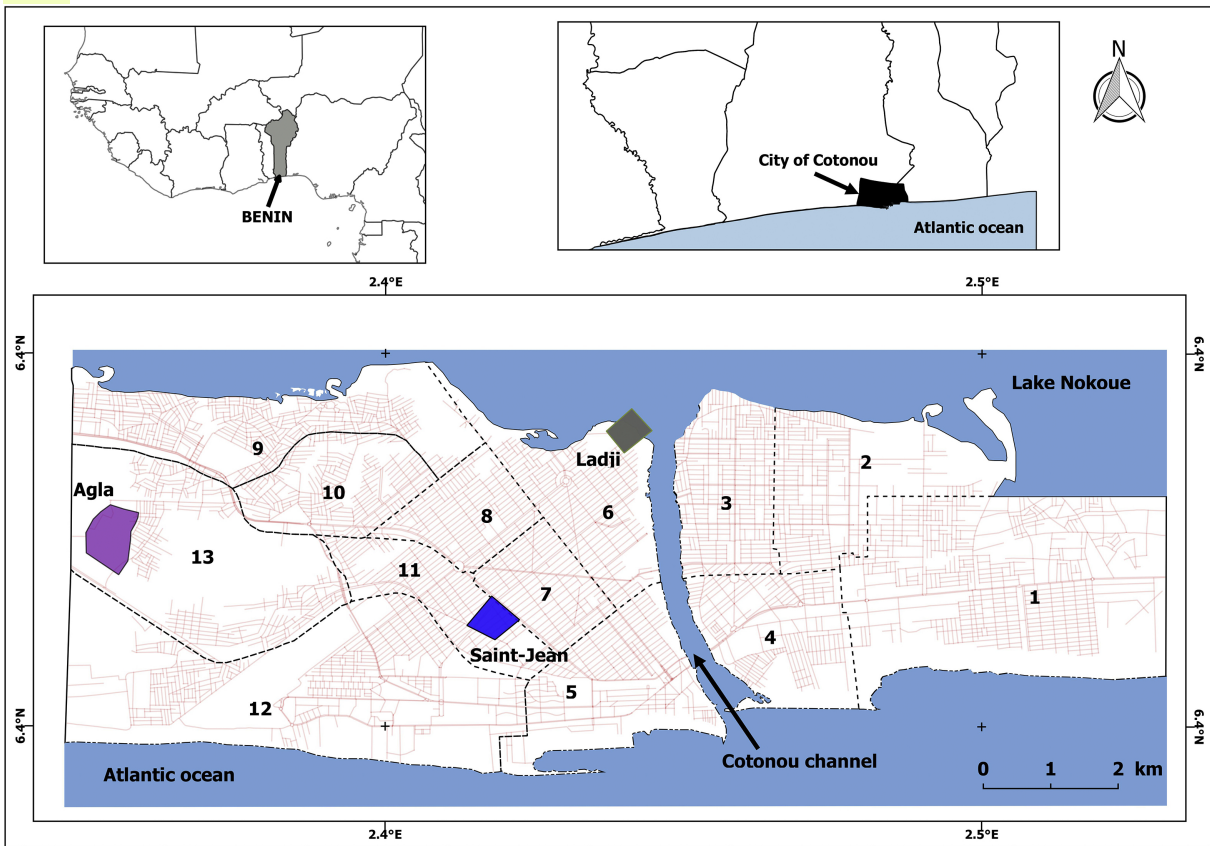


Figure 1



Figure 2

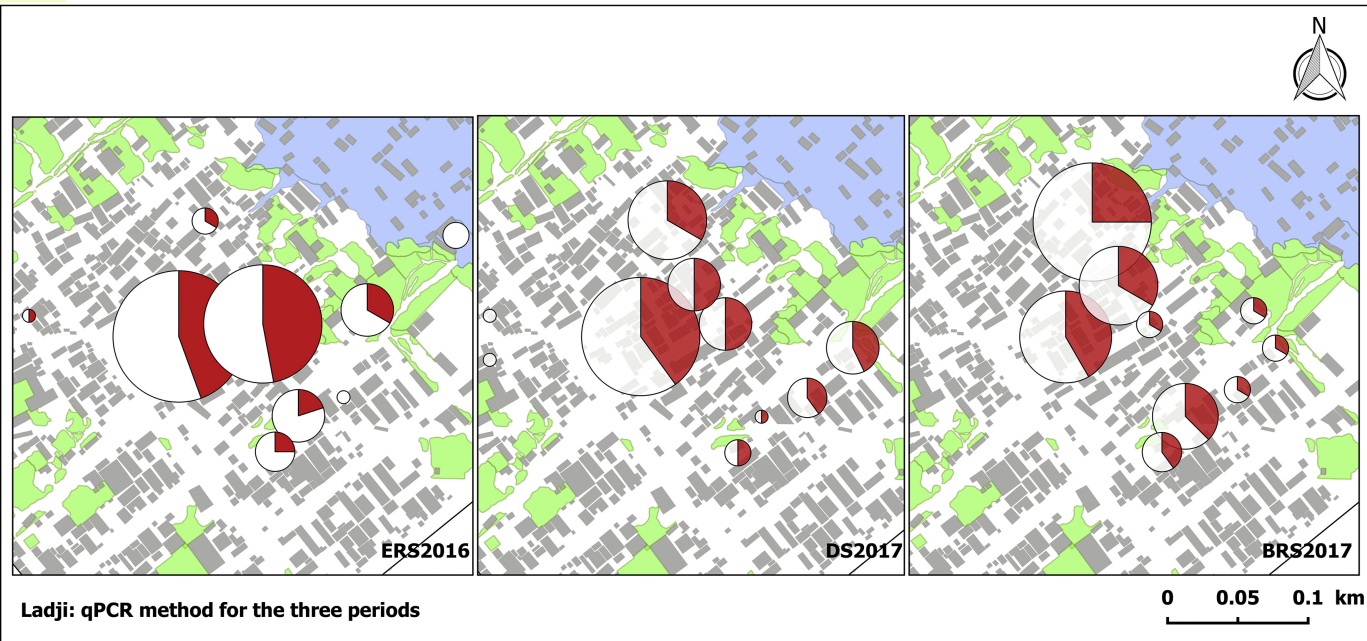


Figure 3



Figure 4

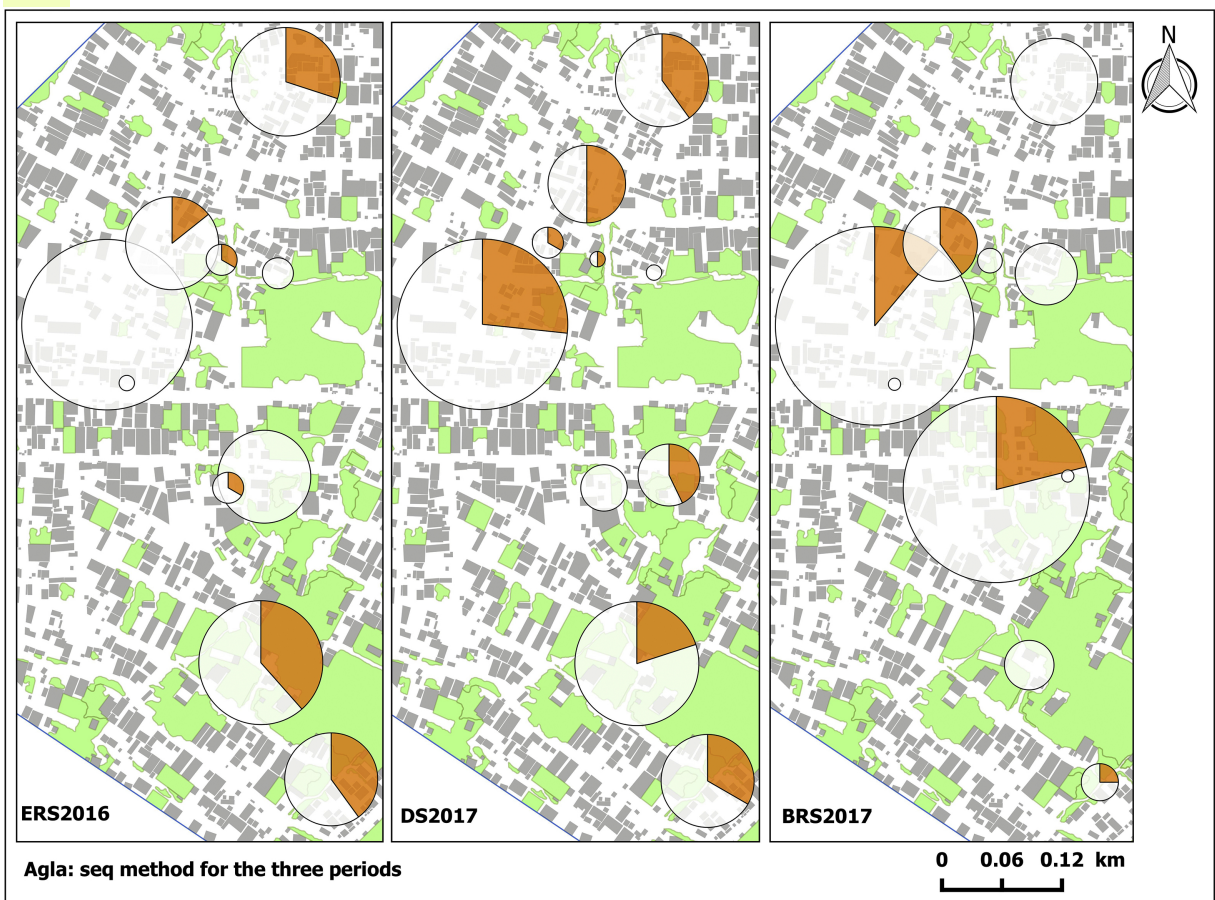
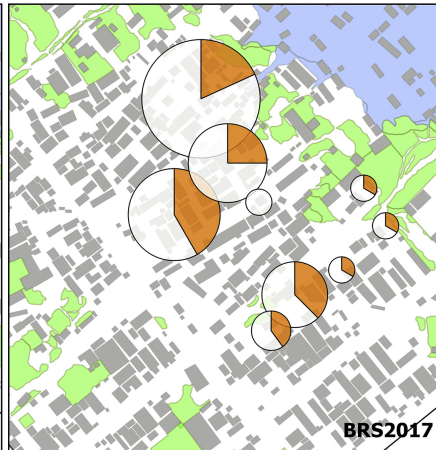
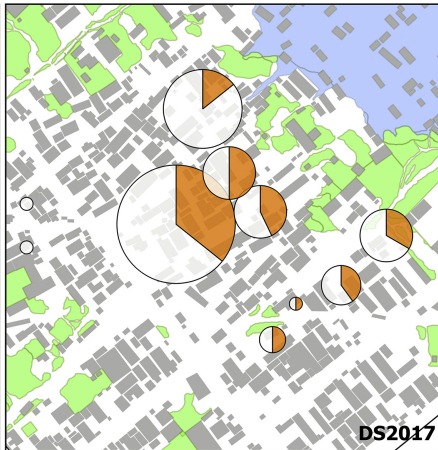
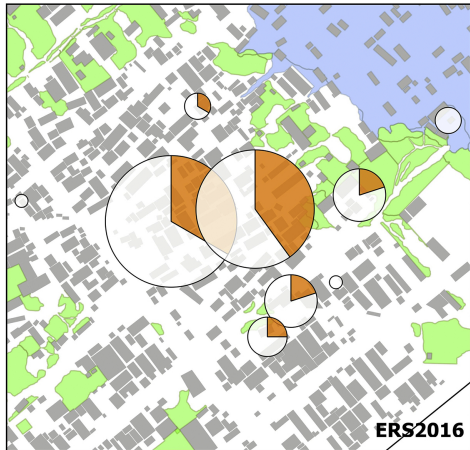


Figure 5



Ladji: seq method for the three periods

0 0.05 0.1 km

Figure 6



Figure 7