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Spatiotemporal variations of the incidence of the fleas (Siphonaptera) on domestic small mammals in the city of Cotonou, Benin.

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ABSTRACT*:*
Objectives: A survey of domestic small mammals and their associated fleas was conducted in Cotonou during the years 2008, 2009 and 2010. The objective of this study was to evaluate the potential role of small mammals in the transmission of anthropozoonosis.
Methodology and Results: A total of 1,402 domestic small mammals were captured in 54 stations using methods following Houémenou (2006). The most abundant small mammals were, *Rattus rattus* (black rat) (63.7%), *Mastomys sp*. (multimammate rat) (11.84%), *Rattus norvegicus* (brown rat) (11.48%) and *Crocidura olivieri* (7.85%). Among these rodents, 364 individuals were found with ectoparasites, and 886 fleas were collected (flea index 0.63), the most common flea being *Xenopsylla cheopis* (rat flea) (97.2%). This species was found in all areas of the city and infested all small mammal species. The Pulicidae prevalence (26%) showed important variations, with relatively low and significantly higher prevalence during the rainy and dry seasons, respectively.
Conclusions and application of findings: Small mammal community in Cotonou was dominated by *Rattus rattus* and fleas by *Xenopsylla cheopis*. Due to the presence of a seaport of paramount importance, Cotonou town was not away from importation by sea transport of zoonotic rats (shelter ships calling at the port of Cotonou, from all continents). To date, in Cotonou there is lacking information on pathogenic agents hosted by the small mammals and their ectoparasites. Therefore, awareness of the authorities on the zoonotic risk of small mammals for the human population is necessary. The authorities of the Port Autonome de Cotonou must increase the screening of ships, which arrive at Cotonou to avoid zoonotic rodent importation. The Ministry of Human Health in Benin must sensitize the populations at high risk of the rodent transmitted diseases. Further studies are needed to detect the different animal reservoirs of these pathogenic agents.

INTRODUCTION

Urban population is growing faster in developing countries than in developed ones (UNDP, 2012). In African cities, urban rapid population growth usually exceeds national and local government capacity for the supply of basic services. This raises associated issues of poverty, unemployment, poor housing, poor or complete lack of sanitation (Firdaus, 2012). Rodents are among the most important reservoirs of zoonotic agents ((Shoukry *et al*., 1991; Meersburg & Kijlstra, 2007)). As an example, Lassa fever, a viral hemorrhagic disease caused by the Lassa arena virus is maintained in the wild – and putatively transmitted to humans- by *Mastomys natalensis* (multimammate rat) (Bausch & Rollin, 1997; Lecompte *et al*., 2006). Another famous illustration is rats and their role in the plague epidemiology, a disease that killed and still kills more people in the World that wars. (Birben, 1975; Bakr *et al*., 1996; Audouin-Rouzeau, 2002). Many cases of plague emergence worldwide was driven by the introduction of synanthropic rats (usually from the genus *Rattus*), along with their associated fleas (such as *Xenopsylla cheopis* in rats). The latter reservoir and vector species can both find acceptable conditions on ships. As such the role of transport, including sea transport, in their dissemination seems pivotal (Biraben, 1975). This makes many harbours across the World at risk from *Yersinia pestis* introduction. As such, Cotonou, main town of Benin, has an international seaport where ships from most continents land. From there, important roads connect Cotonou with the hinterland and surrounding countries such as Burkina Faso, Niger, Chad and Mali. Recently, a survey of small mammals conducted in urban and coastal environments of Benin by (Houéménou, 2006), showed the abundance of *Rattus rattus* (59.8%), *Mastomys sp*. (13.5%), and *C. olivieri* (8.6%). The purpose of this article was to show results about their fleas as a preliminary step towards the assessment of the potential role of small mammals of Cotonou city in the transmission of anthropozoonoses.

MATERIAL AND METHODS

Trapping of rodents was conducted following Houéménou (2006) from July to November 2008 and November 2009 to December 2010. The trapping stations were scattered throughout Cotonou and were grouped in two types of environment as:

- Uninhabited areas or none resident putative high risk areas: swamps
- urban environments with four habitat types according to resident population and the degree of sanitary infrastructure expressed in terms of potential risk of zoonoses.

- low risk: comfortable accommodations, modern health facilities often close to the sea;
- Moderate risk: basic sanitation, with latrines and sinks, open gutters. This was the centre of the core city;
- high risk : no adequate health infrastructure, makeshift houses, "wild" dumps, along the lake and Cotonou channel to the shore of the sea;
- Markets and warehouses seaport of Cotonou.

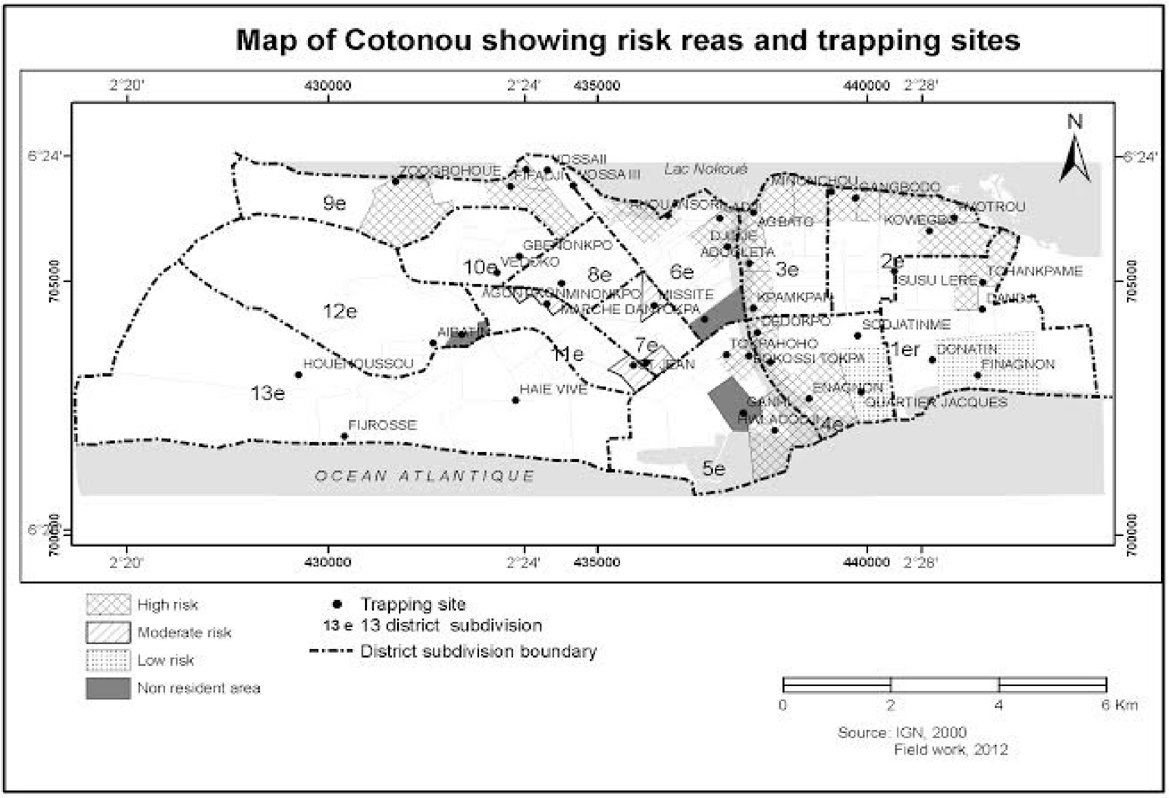

Fig 1: Map of Cotonou showing risk areas and trapping sites

Trapped rodents were anesthetized by placing those in iron bag containing cotton wool soaked with chloroform or ether. Taxonomic identifications were performed based on morphologic traits following De Visser *et al*. (2001). Ectoparasites killed during the rodent or shrew anaesthesia were collected from the bag with a flat forceps, while potentially remaining ones were sampled directly from the fur using a fine toothcomb over a sheet of white paper. These dislodged parasites from each rodent were pooled in collection tubes containing 70% alcohol and numbered in reference to their host. After dehydration in ethanol of increasing concentration, they were mounted between slide and cover slide using Eukitt (R) VWR 24065 or fixed within Hoyer's solution (Fain, 1984). Identification of flea was performed on morphologic grounds under a microscope (Ref Opt B151: 10 X 40) following Hopkins & Rothschild (1953) and Beaucournu & Launay (1990).

Several indicators were then calculated:

- The prevalence was the ratio between the numbers of infected animals vs. the total number of captures.
- The flea index was the average number of flea hosted on a mammalian host species (specific index or for the whole community of infected mammals (General Index).

The sex ratio of flea-bearing animals was tested using the Z-score, while the sex ratio between infected and uninfected individuals was investigated through a $\chi 2$ test. For each host species, the prevalence of ectoparasites was compared between habitats and seasons (months) by a G-test (Goodness of fit test: Sokal & Rolhf, 1981).The distributions frequency of the number of fleas per host species were compared by a nonparametric variance (Kruskal-Wallis) and Kolmogorov-Smirnov (D).

RESULTS

A total of 1,402 small mammals were captured in the 54 stations within Cotonou, with a total trapping effort of 8,340 trap-nights. Overall, the black rat *Rattus rattus* was dominant (N=893) with 63.7% of the whole sample. *Mastomys sp*. (N=166; 11.84%), *Rattus norvegicus* (N=161; 11.48%), *Crocidura olivieri* (N=110; 7.85%) were much less common, while others were rare: *Arvicanthis niloticus* (N=4; 0.29%), *Crocidura* sp. (N=4; 0.29%), *Cricetomys gambianus* (N=6; 0.43%), *Mus domesticus* (N=9; 0.64%), *Gerbilliscus kempi* (N=11; 0.78%),*Taterillus gracilis* (N=14; 1%) and *Dasymys rufulus* (N=24; 1.71%) (Table 1).The black rat was widespread all over the city since it was present in 47 stations out of the 54 (87%) with captures ranging from 1 to 74 (Table 2). Three other species also displayed a large distribution and were detected in more than half of the stations: *Crocidura olivieri* (65%), *Mastomys sp.* (63%), and *Rattus norvegicus* (52%). Among these rodents and shrews, 364 individuals, representing all eleven species, were found to be parasitized by fleas, thus representing an overall prevalence of 26% (Table 1).

Table 1: Prevalence of flea (Siphonaptera) per animal host

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Species	number of captures	nb of animals Hostt of flea	specific prevalence	nb of flea	nb of flea/animal host
C. gambianus	6	1	16.7	9	9
C. olivieri	110	25	22.7	62	2.48
Mastomys sp.	166	54	32.5	157	2.91
R. norvegicus	161	55	34.2	146	2.65
R. rattus	893	228	25.5	510	2.24
T. gracilis	14	1	7.1	2	2
Total	1,350	364	27.0	886	2.43

On the eleven species, only six were parasitized by fleas, with *R. norvegicus* being the most infested (N=55 infested individuals out of 364 examined; 34.2%). Prevalence's in the five other species were as follows: 32.5% (N=54) in *Mastomys* sp, 25.5% (N=228) in *R. rattus*, C. olivieri 22.7% (N=25), C. gambianus 16.7% (N=1) and T. gracilis 7.1% (N=1). Five species were not parasitized by fleas: *A. niloticus, Crocidura sp., G. kempi, D. rufilus* and *Mus domesticus*. Small differences in prevalence were observed: brown rats were more infested than other mammals (partial Gtest1ddl = 4.03, $p < 0.05$) (Table 1). The number of individuals parasitized by Pulicidae was relatively constant for the four most abundant small mammals (KW = 6.92 ns). However, the frequency distribution of the number of fleas in *Mastomys* was significantly different from that observed in the other host species (KS: D (*Crocidura*) = 0.242; D (*R. norvegicus*) = 0.195; D (*R. rattus*) = 0.170; p << 0.01): 30% of *Mastomys* individuals hosted one flea, while half of the other mammal host did so (Fig. 2).

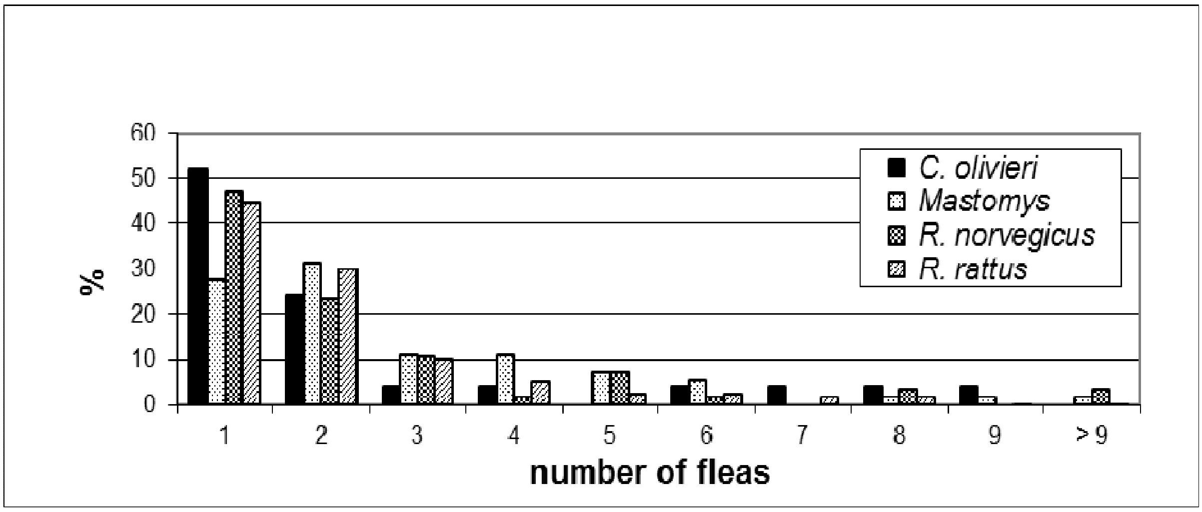

Fig. 2: Frequency distribution of number of flea per host

Animals infested by fleas were detected in 40 of the 54 stations (74%). The highest percentages of infested animals were found in Midombo (56%) and Ganhi (48%), followed by Abokicodji (44%) and and 43%.Percentages were poorly informative in some sites where captures were too rare (N<11 animals). In uninhabited areas, the prevalence of Siphonaptera was minimal (1.3%). It was moderate (12.5%) in low-risk ones, but was much more important in other areas like markets (32%) (Table 2).

Table.2: Prevalence of Siphonaptera in different sitesper risk

	nb of animal host			G test partial (ddl = 1)	
Areas	74	1	1.35	36.13	
Low risk	56	7	12.50	8.66	p < 0.001
Moderate risk	300	83	27.67	0.59	ns
High risk	849	236	27.80	1.35	ns
Markets	116	37	31.90	1.98	ns

The overall flea index was 0.63 (886/1402), but the infestation was significantly greater in Mastomys and *Rattus norvegicus* (p <0.005,) while it was lower in *R. rattus* and *T. gracilis.* As far as specific diversity is concerned, the community was particularly poor: only three species of flea were identified: *Xenopsylla cheopis*, *Xenopsylla brasiliensis* and *Ctenocephalides felis strongylus*. *X. cheopis* (N=861) represents 97.3% of all fleas found. It infested black rats in 40 stations out of 54, achieving the highest prevalence in the Abokicodji station of infested animals. Its main host, *Rattus rattus*, was present in 47 trapping sites, or all stations in the urban core environment. As such, the 40 sites where this flea was present were predominantly characterized by human settlements and the presence of garbage. Its presence would be strongly dependent on these two factors. *Xenopsylla brasiliensis* represents only 2.7% of all fleas (N=24) when *X. cheopis* (N=861) represents 97.3%. According to Audouin-Rouzeau, 2003. *X. brasiliensis* was the main flea in rural areas rather than urban centres. One cat flea, *Ctenocephalides felis strongylus* was collected on a Norway rat captured in Wlacodji. The frequency of infested mammals showed significant changes during the year (Fig.3).

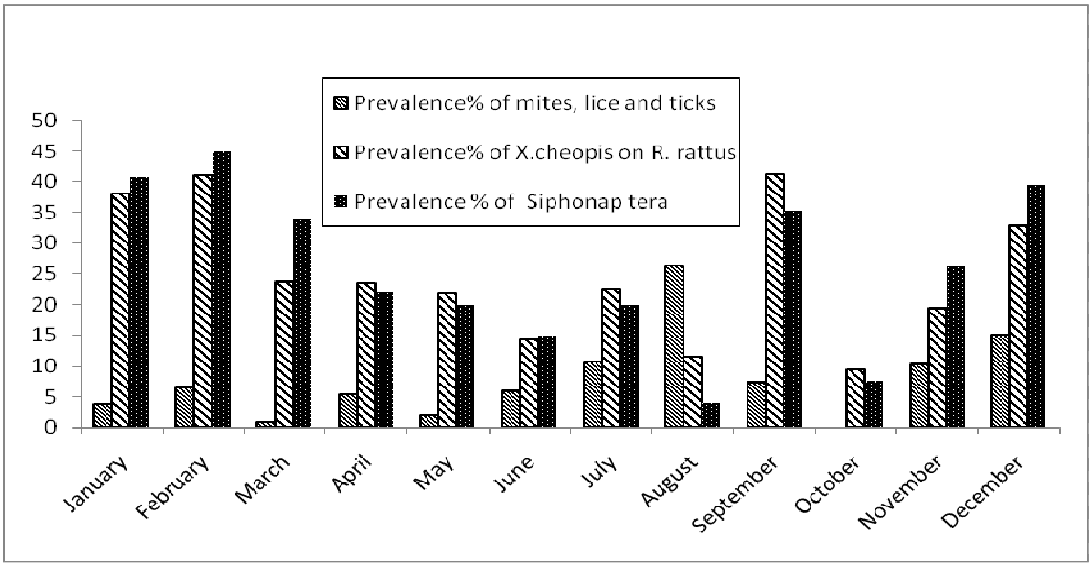

Fig 3: Monthly prevalence of mammalian ectoparasites in Cotonou

The prevalence of flea showed a peak in December, January and February and then decreased from March to June for back only in July, August and fall, shows a peak in September. In October, prevalence drops sharply and then rises again in November (G = 102.44; p <<< 0.001).

DISCUSSION

Surveys of urban rodent diversity are quite scarce, especially in African cities (Garba, 2012). The capture of a large number of *R. rattus* was consistent with its wide distribution in the World (Vanasco *et al*., 2003; Wilson & Reeder, 2005) and with its attested presence in other African cities like Alexandria and Cairo, Egypt (Morsy *et al*., 1988; Khalid *et al*., 1992), Harare, Zimbabwe (Zimba *et al*., 2011), Kinshasa, RDC (Laudsoit, 2004), Niamey, Niger (Garba, 2012) or Makurdi, Nigeria (Omudu & Ati, 2010). This result is consistent with the hypothesis that the black rat is able to better adapt to tropical climatic and environmental conditions than the brown rat, *R. norvegicus* (Feng & Himsworth, 2013). The relatively high number of *Mastomys sp*. and *Crocidura* spp. was supported by previous studies in Africa (Ratzooman, 2012). Several authors have sampled small mammals and their ectoparasites in different African cities, including Alexandria (Morsy *et al*., 1988), Cairo (Khalid *et al*., 1992.), Kinshasa (Laudisoit, 2004), Cotonou (Houémènou, 2006; this study), Makurdi (Omudu & Ati, 2010) and Harare (Zimba *et al*., 2011). These authors calculated parasitic or Pulicidae prevalence as well as a series of indices: parasitic rate, flea index, among others. These results show a high variability between studies. The ectoparasitic prevalence varies between 10% (Morsy *et al*., 1988) to over 60% (Omudu & Ati, 2010). In the same manner, the overall flea index varies from 0.08 (Omudu & Ati, 2010) to 6.1 in urban peripheries of the upper valley of the Egyptian Nile (Maher *et al*., 1974). In Cotonou, the overall flea index was 0.63, significantly more important than that of Kinshasa (0.28; Laudisoit, 2004) or in the city of Makurdi, Nigeria (0.08; Omudu & Ati, 2010). In these cities, all small mammals, rodents and shrews, infested with fleas were hosts of *Xenopsylla* (Laudisoit, 2004; Omudu & Ati, 2010; Zimba *et* al., 2011) like in this study (with the exception of the single cat flea). In addition, in all these case-studies, the *Rattus rattus* and *Xenopsylla* (*cheopis* and *brasiliensis*) combination seems constant: (Benin : this study; Congo: Laudisoit, 2004; Egypt: Maher Ali *et al*., 1974; Aboul Ela *et al*., 1987; Bakr *et al*., 1996; Khalid *et al*., 1992; Morsy *et al*., 1988; Nigeria, Omudu & Ati., 2010; Zimbabwe, Zimba *et al*., 2011). This pattern shared between African cities may be extended further, for instance in Asia (India: Achutan & Chandrahas, 1971; Philippines: Mercado, 1981). *X. cheopis* was considered urban flea by Beaucournu (1999). Our study in Cotonou is consistent with their suggestion. However, the global index of *X. cheopis* in Cotonou was relatively small, 0.60 per rat (*R. rattus* and *R. norgevicus*).This value appears much less than 5, above which one considers that an outbreak of plague can occur (Aboul-Ela *et al*., 1987).The seasonal variation of flea abundance was assessed by different authors. In India, the prevalence of rat-borne *X. cheopis* from rural Kolar was maximum in August, September and October with a peak in August while it was minimal in April, May and June (Achutan & Chandrahas, 1971). In the Cairo area, Khalid *et al*. (1992) and Bakr *et al*. (1996) found that the flea index was higher in spring but close to null in winter. In the Upper Nile region, Maher Ali *et al*. (1974) found a similar pattern in the suburban environment of cities and the cultivated areas (maximum in spring and minimum in autumn), whereas in semi-arid environments, the flea index falls from June until October. However, Laudisoit (2004) reported that in the tropics, the peak abundance of *X. cheopis* was observed during the coldest season. Closer to Benin, Ugbomoïko & Obiamiwe (1991) found that, in Ekpoma (southern Nigeria), the seasonal rains positively influence adult fleas' abundance In Tanzania, in Lushoto District, Njunwa *et al*. (1989) recorded the highest flea index from December to May. A few years later in Lushoto district, Makundi (1999) reported that "the intensity of fleas (flea index) was highest in December / January and rapidly declined in April / May concomitantly with the peak of the rainy season. It was further observed that fleas were more abundant on rodent hosts when ambient temperatures were 22–26°C during November to April. Our results seem congruent with these findings. The lack of work on the subject in the sub-region of western Africa made the comparison of results difficult.

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