High Prevalence of *Rickettsia typhi* and *Bartonella* Species in Rats and Fleas, Kisangani, Democratic Republic of the Congo

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Abstract. The prevalence and identity of *Rickettsia* and *Bartonella* in urban rat and flea populations were evaluated in Kisangani, Democratic Republic of the Congo (DRC) by molecular tools. An overall prevalence of 17% *Bartonella* species and 13% *Rickettsia typhi*, the agent of murine typhus, was found in the cosmopolitan rat species, *Rattus rattus* and *Rattus norvegicus* that were infested by a majority of *Xenopsylla cheopis* fleas. *Bartonella queenslandensis*, *Bartonella elizabethae*, and three *Bartonella* genotypes were identified by sequencing in rat specimens, mostly in *R. rattus*. *Rickettsia typhi* was detected in 72% of *X. cheopis* pools, the main vector and reservoir of this zoonotic pathogen. Co-infections were observed in rodents, suggesting a common mammalian host shared by *R. typhi* and *Bartonella* spp. Thus, both infections are endemic in DRC and the medical staffs need to be aware knowing the high prevalence of impoverished populations or immunocompromised inhabitants in this area.

INTRODUCTION

The last two decades have been characterized by the emergence or re-emergence of various diseases on the world epidemiological scene, among which are a series of endemic zoonotic infections, most of them being rodent or vector-borne.¹ Endemic foci do usually refer to rural settings, but the focal distribution of pathogens may occur in urban areas offering a mosaic of habitats suitable to hosts,² vectors,³ pathogens survival,⁴ and exposed human population.⁵ At different spatial and temporal scales, the anthropogenic modifications directly and indirectly play a role in the host and vector dynamics and act on the probability for (re)emergence of zoonotic diseases. Factors leading to an increase in the incidence of illnesses caused by zoonotic bacteria in urban areas include societal changes and intrinsic components of the natural history of these organisms that favor their survival in cities.⁶ It is thus crucial to understand interactions between land change, ecology, and dynamics of potential vectors, animal and human hosts constituting those so-called “pathogenic landscapes.”⁷ Kisangani is a major city that consists of a mosaic of landscapes or microhabitats that create an array of ecological niches, which in turn may increase the risk for specific pathogen-carrying hosts and vectors to establish. However, the problem of densely populated, major metropolis of sub-Saharan Africa has so far been overlooked.⁸

Typically, *Rickettsia* and *Bartonella* species are emerging zoonotic bacteria that occur in humans, rodents, and their ectoparasites^{9–11}; and they are increasingly reported worldwide. Rickettsioses are diseases caused by infection with obligate intracellular bacteria (genus *Rickettsia*) many of which are pathogenic for humans, and all are vector-borne (lice, ticks, fleas, mites). The *Rickettsia* species described fall into four groups of which the typhus group (TG), comprises *Rickettsia typhi* and *Rickettsia prowazekii*, the spotted

fever group (SFG) comprises 24 rickettsial species including *Rickettsia felis*, *Rickettsia canadensis* group, and *Rickettsia bellii* group.¹²

Likewise, bartonellosis are diseases caused by infection with the facultative intracellular bacteria. Species of the genus *Bartonella* are associated with an increasing array of human diseases, among which bacillary angiomatosis and peliosis hepatitis in immunocompromised patients.¹³ Mammalian reservoirs of *Bartonella* include small carnivores and rodents,¹⁴ their transmission rate depends on the *Bartonella* strain, host, and specific vector. Historical data on rickettsiosis and bartonellosis in the Democratic Republic of the Congo (DRC) (former Zaire) are fragmentary, but serological tests have reported *Rickettsia conorii*, *Rickettsia prowazekii*, and *R. typhi* as early as the 1950s.^{15,16} These data are currently obsolete, and there is a real urgency to estimate the identity and prevalence of *Rickettsia* and *Bartonella* species in the DRC. Moreover, recent surveys in DRC revealed that 1) in the Ituri district (north east of the Orientale province), Congolese patients (4.5%) were seropositive for *Bartonella henselae*, *Bartonella quintana*, or *Bartonella clarridgeiae*,¹⁷ but not for *Rickettsia*¹⁸; that 2) domestic arthropod harbor *Rickettsia* spp. and *Bartonella* spp.¹⁹; that 3) rodents harbor *Bartonella* spp. closely related to *Bartonella elizabethae*, or *Bartonella tribocorum*²⁰; and 4) in Kinshasa, that *Rickettsia africae* and *R. felis* circulate in arthropod vectors.²¹ A preliminary study was carried out in Kisangani, where the relatively recent stability has changed the profile of the city into a fast-growing economical center in eastern DRC with 1,310,587 inhabitants.²² It seems likely that the increasing numbers of refugees or migrants have augmented the pressure on the ecosystems surrounding Kisangani. Because of this intensifying human encroachment into natural ecosystems, the risk of importing exogenous zoonotic pathogens could raise.^{23,24} Moreover, traders and consumers of the central market claimed to regularly consume rodents captured in their own homesteads (Falay A, unpublished data). Finally, because of its fluvial connection with Kinshasa, the potential influx of rats infested with *Xenopsylla cheopis* fleas, a known vector of murine typhus (*R. typhi*) is a true concern.²⁵ Our survey pursued three major objectives: 1) to estimate the

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prevalence and identity of *Bartonella* spp. and *Rickettsia* spp. in *Rattus* spp. populations of Kisangani town; 2) to identify the potential vectors and/or carriers of those zoonotic agents; and 3) to elaborate realistic recommendations to improve the monitoring and control of urban rat populations and their ectoparasites in Kisangani, which could eventually be implemented throughout the DRC and elsewhere.

MATERIAL AND METHODS

Sites and habitats. In collaboration with a team of zoologists from the Faculty of Sciences (Kisangani University, DRC) a rodent and flea survey was conducted in several urban and peri-urban areas of Kisangani town in April and June 2011. Kisangani (0°30'N; 25°11'E) is the principal city of the Orientale province, mainly situated between the Congo River right bank and the Tshopo River. It covers 1910 km² and consists of six administrative districts (Makiso, Tshopo, Kisangani, Mangobo, Kabondo, and Lubunga) and an estimated 80% of the population lives below the poverty line. The economy of the city is primarily based on artisanal mining, "small trade businesses" and agriculture. The sampling sites were selected in relation to the occurrence of rats observed or reported by people and to their potential threat to public health; namely, a slaughterhouse (SH), the central market (CM); the International Aeronautic Transit (IAT) market (IM), and the Tshopo market (TM). The slaughterhouse (0°32'N, 25°10'E) is located near the left bank of the Tshopo river in Mangobo district, a suburban-rural neighborhood where the main income is based on agriculture. This site is situated in a savannah habitat, scattered with corn or cassava fields. The IAT market (0°30'N; 25°10'E) is located in the Makiso district, at the city's secondary port where merchants from the surrounding villages trade and reside temporarily. The CM (0°30'N; 25°11'E), located in Makiso district, is surrounded by shops, warehouses (rice, wheat flour, cassava or corn flour, fry fish, smoked bushmeat, etc.) and homes. Because of its strategic position, this market provides the majority of the sylvatic products (including bushmeat) sold in town.²⁴ The TM (0°31'N, 25°11'E), consists of open air stalls and warehouses and is situated in the popular Tshopo district (100,000 inhabitants). Hygienic conditions are on the whole poor (lack of proper drainage of sewage, garbage disposal, or septic tanks) for cohabiting families living on and around the market places.

Rodent trapping. Rodents were trapped with Tomahawk collapsible live traps (dimensions 490/178/173 mm, $N = 10$, 6 nights/site, 60 trap nights/site) baited with salted and smoked fish. Traps were set at sunset and checked the next morning. The location of each positive trap was recorded using a global positioning system (GPS) (Garmin 60Cx, Southampton, Hampshire, UK). Rodents were taken to the Faculty of Sciences (University of Kisangani), where they were anaesthetized with isoflurane and combed above a white tray to collect ectoparasites. Every rat was identified using morphological characteristics, and were given a unique label and stored in 70% ethanol. For each rat, a series of external measurements was recorded and biopsies were stored in 96% ethanol. Ectoparasite species were identified at the University of Antwerp, pooled per host, flea species, day and location of trapping, and crushed in dry ice before DNA extraction.

Genetic analysis. Total DNA was extracted from samples (*Rattus* spp. organs: spleen, heart, liver and left kidney, and flea pools) using the commercial kit QIamp DNA mini kit instructions (Hilden, Germany) according to the manufacturer's recommendations. The DNA was eluted in 100 µL of buffer AE.

Detection of *Bartonella* in host samples. Regular polymerase chain reaction (PCR) was performed using the primers 1400F and 2300R²⁶ targeting 852 base pairs (bp) of the *Bartonella* DNA polymerase beta subunit (*rpo* B).²⁶ A sample was considered as positive when a 900 bp fragment was visible. Positive controls consisted of rat DNA found positive for *Bartonella* sp. and *B. elizabethae* in previous studies.^{20,27}

Detection of *Rickettsia* in host samples and flea pools. The PCR assay was performed using the primers 120-M59F and 120-807R targeting 833 bp of the *ompB* *Rickettsia* protein-encoding gene.²⁸ Amplifications were performed in 20 µL reaction volume containing 10 µL of PCR mix (Qiagen multiplex PCR kit no. 206143), 0.5 µL of each primer (10 µM), and 2 µL of total DNA. A sample was considered as positive when a 850 bp PCR product was visible. Positive controls consisted of flea DNA from domestic fleas' specimen extracted *in toto*, and collected in locations (households), which had been previously found positive for *Rickettsia* sp. in a former study.¹⁹

Genetic identification of rodent, *Rickettsia*, and *Bartonella* species/strains. For each rat, DNA was extracted from a mixture of the four organ biopsies to 1) confirm the host species identity using mitochondrial DNA cytochrome *b* sequencing using L7 and H6 primers²⁹; obtained sequences were compared with known sequences of the *Africanrodentia* database (<http://projects.biodiversity.be/africanrodentia/>), 2) detect the presence and estimate the prevalence and strain identity of *Bartonella* spp. and *Rickettsia* spp. To identify the *Bartonella* spp. and *Rickettsia* spp. strains, positive PCR products were purified and sequenced by VIB Genetic Service Facility (University of Antwerp, Belgium) using the same primers as used for the PCR. The *Bartonella* and *Rickettsia*-positive DNA or PCR products from rodents and fleas were sent to URMITE, Marseille, France. All samples were screened for all spotted fever group rickettsiae (SFGR) using real-time qPCR targeting a fragment of *gltA* gene (RKND03 system),³⁰ and by *R. typhi*-specific qPCR targeting a fragment of RTB9991CWPP_01310 gene coding for a hypothetical protein.³¹ The DNA *R. montanensis* and DNA *R. typhi* were used as positive controls. The concentration of rickettsial DNA is estimated through the number of cycles that is required before the fluorescence signal intensity exceeds the detection threshold (Ct). The *Bartonella*-positive DNA samples were tested by qPCR targeting an internally transcribed spacer (ITS).³² Sequences were checked and aligned using MEGA 5.05. Sequences were compared with other *Bartonella* spp. and other *Rickettsia* spp. using the basic local alignment search tool (BLAST, NCBI) algorithm. During the checking process, clear double peaks (distinct from the baseline noise) were visible on the sequencing chromatograms of four of the flea samples. These may be the result of mixed infections or—caused by the pooling of several flea specimens—to the mixing of several specimens each being infected with a different strain of *Rickettsia*.

Statistical analysis and models. To analyze the correlates of host and environmental factors with the probability that an individual host was infected with either *Bartonella* spp.

or *Rickettsia* spp., we used a generalized linear model with binomial family and a logit link to construct a multiple logistic model in R. The generalized linear model was fit to relate the logit of either *Bartonella* spp. (2 levels, infected or healthy) or *Rickettsia* spp. (infected or healthy) occurrence to host species (2 levels: *Rattus rattus* or *Rattus norvegicus*), month (2 levels: April or June), sampling sites (4 levels: SH, IM, CM, TM), *X. cheopis* infection (in a first step, flea number, continuous, and in a second step, 2 levels: infected versus not infected), mite infection (2 levels: infected versus not infected), and weight (continuous). Model selection was based on the akaike information criterion and likelihood-ratio tests and we used a top-down protocol.³² Statistical significance was declared at *P* values of lower than 0.05. In summary, the generalized linear starting model included the following sets of variables: *Bartonella* infection or *Rickettsia* infection, host species, month, locality, *X. cheopis* infestation, mite infestation, and weight. The final model over *Bartonella* was finally run on a reduced data set containing only two sites (CM and IM), and the factor species ($\chi^2 = 141.5$, degrees of freedom [df] = 6, $P < 0.0001$), whereas the *Rickettsia* model included the factors species and weight ($\chi^2 = 7.8$, df = 1, $P = 0.0052$).

RESULTS

Rat and flea distribution. A total of 240 traps (60 per site) were placed during the study period (from 12/01/11 to 12/06/11) with a daily variable trapping success, ranging from 100% (IM) to 0% in TM and SH site. In total, 126 sexually mature *Rattus* spp. were trapped; the sample consisted of 106 *R. norvegicus* and 20 *R. rattus*. The oriental rat flea *X. cheopis* ($N = 188$) was collected off 56 *R. norvegicus* ($N = 164$) and 7 *R. rattus* ($N = 24$), whereas *Xenopsylla brasiliensis* ($N = 5$), the African rat fleas was collected off only two *R. norvegicus*. Table 1 summarizes the number of rodents trapped per site and the number of fleas collected off them.

In summary, the predominant host species and flea species were *R. norvegicus* (84%) and *X. cheopis* (97.4%). For a similar trapping effort, significantly more *Rattus* spp. were caught in the IAT market (50%) and CM (37.3%) than in the SH (4.8%) and TM (7.9%) area ($\chi^2 = 38.7$, df = 3, $P < 0.0001$). In all four sites, *R. norvegicus* was significantly more often caught than *R. rattus* ($\chi^2 = 39.3$, df = 3, $P < 0.0001$).

Prevalence of *Bartonella* and *Rickettsia* species in Kisangani *Rattus* species. In the sampled rats, the overall *Bartonella* spp. and *Rickettsia* spp. prevalences were 16.7% and 12.7%,

respectively. We trapped fewer *R. rattus* than *R. norvegicus*, and the proportion of *R. rattus* infected with *Rickettsia* (20%) or *Bartonella* (25%) was similar to the prevalence of *Rickettsia* (11.3%) or *Bartonella* (15%) in *R. norvegicus*. No *Bartonella*-positive animals were found in SM and TM sites, whereas *Rickettsia*-positive rodents and fleas were detected in all four sites. However, the model including overall infection with *Bartonella* spp. and host species identity—removing the site because of low capture rate—revealed that the probability of being infected with *Bartonella* spp. was significantly higher for *R. rattus* ($P = 0.5$) than *R. norvegicus* ($P = 0.16$) ($\chi^2 = -1.56$, df = 1, $P < 0.001$). Four rodents (2 *R. norvegicus* and 2 *R. rattus*) were coinfecting with *Bartonella* spp. and *R. typhi* and all came from the IAT market near market along the Congo River. In the *Rickettsia* model, the only relevant factor, namely weight, was significantly and negatively correlated across species, to the probability of having *Rickettsia* spp. ($P = 0.98 + 0.99 \times \text{weight}$; df = 1, $P = 0.02$).

Genetic diversity of *Bartonella* DNA sequences. All 17 *Bartonella*-positive rodent samples by standard PCR were confirmed positive by qPCR targeting an internally transcribed spacer with the mean Ct value with SD 30 ± 2.1 (URMITE). Out of 11 readable *Bartonella* sequences (± 800 –852 bp) retained (six sequences discarded because of poor quality), four of them were similar (96–100% homology) to the reference strain *Bartonella* sp. 1-1C (GenBank EU551156.1), a *Bartonella rochalimae*-like strain described from *R. norvegicus* in Taiwan. Interestingly, whereas *Bartonella* sp. was more prevalent in *R. rattus*, the four *B. rochalimae*-like sequences from this study were only found in *R. norvegicus* adult males. Four sequences (± 825 bp) were similar with 97–100% homology to an uncultured *Bartonella* sp. isolate RRB047N (GenBank GU143495) and one sequence showed 100% homology with the reference strain RRB170N (GenBank GU143502), both detected in *R. rattus brunneusculus* from Nepal. One sequence shared 100% homology with the *Bartonella queenslandensis* strain (GenBank EU111790) identified in Australian rats belonging to the genera *Melomys* and *Rattus*. *Bartonella elizabethae* was identified in a *R. norvegicus* (CM), showing 100% homology with the reference strain (GenBank AF165992).³¹

Identification of *Rickettsia*. Out of 18 flea pools, 13 (72.2%) were positive for the targeted *ompB* fragment of *Rickettsia* spp. *Xenopsylla cheopis* was the only flea species infected with *Rickettsia* spp. The positive controls consisted of one *Pulex irritans* and one *Ctenocephalides felis strongylus* from Ituri district,¹⁹ and showed 99.8% (787 of 788) homology with *Rickettsia* sp. R14 (GenBank HM370113) and *Rickettsia* sp. cf9 (GenBank DQ379483) detected in fleas from India and the United States, respectively, and only 94% (727 of 777) similarity with *R. felis* (GenBank GQ329879) suggesting that this is a *Rickettsia felis*-like bacterium. In the study of Sackal and others,¹⁹ it was identified as *Rickettsia felis* by real-time multiplex PCR assay suggesting that the molecular system used was not specific. The positive samples in this study were identified as *R. typhi* showing 100% homology with the reference strain *R. typhi* str. B9991CWPP (GenBank CP003398). Similarly, in the readable sequences obtained from *R. norvegicus* and *R. rattus* organs, all *Rickettsia* identified were showing between 98% and 100% homology with the *R. typhi* str. B9991CWPP (GenBank CP003398). All *Rickettsia*-positive samples were confirmed positive by *R. typhi*-specific qPCR.³¹ In the *R. typhi*-specific qPCR, the mean Ct value in rodent and flea DNA

TABLE 1

Site surveyed, trap success, number of *Rattus* sp. caught, number of *X. cheopis* fleas, and *X. cheopis* flea index on *Rattus norvegicus* and *Rattus rattus**

Site	Trap nights	N <i>Rattus</i> sp.	N <i>XcRn</i>	FI <i>Rn</i>	N <i>XcRr</i>	FI <i>Rr</i>
Slaughterhouse	60	4.8%	2	0.0	4	0.8
Central market	60	37.3%	45	2.3	2	1.5
IAT market	60	50.0%	56	1.0	7	0.7
Tshopo market	60	7.9%	3	1.3	7	1.9
Total	240	126	106	1.5	20	1.2

Trap success: number of positive traps; Number of *Rattus* sp. caught: N *Rattus* sp.; Number of *X. cheopis* fleas: N *XcRn*.

*Flea index: number of fleas per host.

Flea index on *Rattus norvegicus*: FI *Rn*.

Number of *X. cheopis* fleas: N *XcRr*.

Flea index on *Rattus rattus*: FI *Rr*.

IAT = market: the International Aeronautic Transit.

specimens with standard deviation ($\pm$ SD) was 31.53 ± 3.60 and 26.03 ± 7.94 , respectively. All these DNA samples tested for SFGR-specific qPCR were negative.

DISCUSSION

In this study, we report a high prevalence of *Bartonella* spp. (17%) with at least five genotypes, and *R. typhi* (13%) with a single strain, in a small urban rat population in the city of Kisangani. This study also draws attention to the relatively high prevalence of *R. typhi* DNA in *R. rattus* (20%), *R. norvegicus* (11%), and in fleas (72%, 13 of 18 pools) in the country. The prevalence of *R. typhi* in fleas and rodents DRC is one of the highest from the world. In the last years, *R. typhi* was identified in 10.8% of *X. cheopis* pools (4 of 16) in Indonesia,³³ in tissues from three rats and in 10% of fleas from each animal in California,³⁴ in 4% *X. cheopis* and 6.6% in *Leptopsylla segnis* in Cyprus,³⁵ and in 3.2% flea pools in Korea.³⁶ The flea remains infected with *R. typhi* for life with good fitness and the horizontal and vertical transmission increase the infected populations in this endemic area.³⁷ Moreover, the rapid spread of flea-borne pathogens to human populations is caused by the frequent feeding behavior and extraordinary mobility of fleas, and to the abundance of cosmopolitan rat species, that once were infected by *R. typhi*, remain infective for life, whereas their lifespan and reproductive capacity are unaffected by the infection.³⁷ This high prevalence of *R. typhi* may have serious public health consequences. Usually, most patients presented mild illness with high fever, headache, chills, and nausea.^{31,39} The rash often appears a week after the onset of fever and is discrete; however, it is not always found. In endemic areas, murine typhus was identified in 10% of patients with undifferentiated febrile illnesses⁴⁰; although, sometimes severe complications can occur (neuropsychiatric disorders, renal, hepatic, pulmonary and cardiac dysfunction) with < 5% mortality rate in large series.³⁸

Bartonella elizabethae, which causes endocarditis was previously identified in rat samples in DRC,²⁰ but *B. queenslandensis*, *Bartonella* sp. 1-1C and 2 uncultured *Bartonella* spp. with unknown pathogenicity were identified first in rat samples. No *Bartonella* spp. were detected in fleas from Kisangani, however *B. clarridgeiae*, *B. vinsonii*, *B. rochalimae*-like, and a new *Bartonella* genotype were previously¹⁹ reported in fleas collected from rural areas of DRC. The circulation of several *Bartonella* species may have a high risk in impoverished populations or immunocompromised inhabitants of the neighborhoods near our sample sites. The prevalence of human immunodeficiency virus (HIV) patients in Kisangani in 1999 was 6%, and among sex workers—for the country, between 1985 and 1997—was 25.4–38%.⁴¹ To date, no human case of bartonellosis has been reported for the DRC, or Kisangani in particular, but evidence of the recent exposure to *Bartonella* was established in patients from the Ituri district in the North of the same province.¹⁷ We found four co-infections with *Bartonella* spp. and *Rickettsia* spp. in rat specimens and recently, the < 1% co-infection with *Bartonella* spp. and *R. felis* was described in fleas,¹⁹ suggesting a common vector and mammalian host shared by both pathogens.

The incidence and prevalence of human rickettsiosis and bartonellosis are probably underestimated in DRC. There are three major reasons for this situation. First is the lack of awareness for those emerging and neglected vector-borne

and rodent-borne infectious diseases. Second, the difficulty to diagnose them because of the high diversity of the clinical manifestations and symptoms, and finally the lack of modern diagnostic tools, often combined with an unreliable electricity supply and the collapse of the local health infrastructure. In the University Clinics of Kisangani for example, the burden of emerging or neglected diseases is unknown and their epidemiological records revealed that 17% of the admitted patients are never given a proper diagnostic (University Clinics of Kisangani; Falay, unpublished). As a consequence, patients do not receive adequate treatment—if any treatment at all—or are treated blindly with a combination of large spectrum antibiotics, which are known to be usually inefficient against *Rickettsia* or *Bartonella* infections.

Our results suggest that the urban *Rattus* populations living in Kisangani only carry cosmopolitan fleas (*X. cheopis* mainly), and that the flea burden did not affect the probability of infection. A similar situation—a nearly monospecific flea infestation—has been observed in Kinshasa,³⁰ and along the Congo river (Laudisoit A, unpublished data). In addition, we found that rodent weight was negatively correlated with the probability of being infected with *R. typhi*. The fact that heavier individuals—and probably older individuals—being less likely infected may reflect acquired immunity against *Rickettsia* as a result of their lifelong and constant exposure to the agent. The *rpoB* gene used in this study is one of the most potent genes for *Bartonella* identification.⁴² However, culture techniques and the analysis of other specific genes or genomes should be done to better characterize the *Bartonella* species identified in DRC.

In conclusion, this study has revealed the urgency 1) to address the issue of vector-borne diseases in major cities in the DRC and cities elsewhere in sub-Saharan Africa; 2) to develop local surveillance and control teams consisting of medical personnel and academicians, and 3) to perform interviews and sociological surveys to identify areas, socio-economic conditions, and times of the year where the transmission risk is the highest to develop adequate rodent and flea control policies.

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