

## Urban Leptospirosis in Africa: A Cross-Sectional Survey of *Leptospira* Infection in Rodents in the Kibera Urban Settlement, Nairobi, Kenya

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**Abstract.** Leptospirosis is a widespread but under-reported cause of morbidity and mortality. Global re-emergence of leptospirosis has been associated with the growth of informal urban settlements in which rodents are thought to be important reservoir hosts. Understanding the multi-host epidemiology of leptospirosis is essential to control and prevent disease. A cross-sectional survey of rodents in the Kibera settlement in Nairobi, Kenya was conducted in September–October 2008 to demonstrate the presence of pathogenic leptospires. A real-time quantitative polymerase chain reaction showed that 41 (18.3%) of 224 rodents carried pathogenic leptospires in their kidneys, and sequence data identified *Leptospira interrogans* and *L. kirschneri* in this population. Rodents of the genus *Mus* (37 of 185) were significantly more likely to be positive than those of the genus *Rattus* (4 of 39; odds ratio = 15.03). Questionnaire data showed frequent contact between humans and rodents in Kibera. This study emphasizes the need to quantify the public health impacts of this neglected disease at this and other urban sites in Africa.

### INTRODUCTION

Leptospirosis has been described as the most geographically widespread and prevalent zoonosis in the world.<sup>1–3</sup> It is caused by infection with different serovars of bacteria of the genus *Leptospira*. Human infection can lead to a range of clinical manifestations, from mild or asymptomatic infections to severe, life-threatening illness. Human disease is severely under reported as patients commonly present with non-specific symptoms, such as fever, headache and myalgia, and infection is difficult to diagnose by using either clinical or laboratory diagnostic criteria.<sup>2,4</sup> Consequently, in many parts of the world, the public health impact of leptospirosis is largely undocumented.<sup>5–8</sup> However, when leptospirosis is considered in clinical and epidemiologic evaluations of febrile populations, significant disease burdens are frequently demonstrated.<sup>4,9–11</sup> Data from studies of febrile illness in many tropical regions indicate that leptospirosis can account for considerable proportions of febrile illnesses.

Among patients hospitalized with acute febrile illness in northern Tanzania, 33.3% of 832 patients showed evidence of exposure to leptospires and 8.8% of 453 patients with paired serum samples had acute leptospirosis based on a  $\geq 4$ -fold increase in microscopic agglutination test titer.<sup>4</sup> Similarly, 15.5% of 773 febrile patients presenting at a hospital in Sri Lanka and 16% of patients in Egypt with acute febrile illness had acute leptospirosis based on seroconversion or the equivalent of a  $\geq 4$ -fold increase in titer using an IgM enzyme-linked immunosorbent assay.<sup>10,12</sup>

In the Kibera informal settlement, where this study was conducted, acute febrile illness is a common syndrome, with

an average of 2.7 episodes per person/year for children < 5 years of age and 0.58 episodes for persons  $\geq 5$  years of age based on household visit data.<sup>13</sup> However, the proportion of febrile illnesses that are attributable to leptospirosis at this site is currently unknown. The published data on leptospirosis in Kenya are also limited. The World Health Organization Global Alert and Response System described a laboratory-confirmed outbreak that occurred at two schools in western Kenya in 2004 that involved more than 141 suspected cases and 8 deaths.<sup>14</sup> Several patients assessed during an outbreak of acute febrile illness in northeastern Kenya in 2005 were positive for antibodies against *Leptospira*.<sup>15</sup> Other published data on leptospirosis in Kenya date back to the 1960s and 1970s and describe human cases in several provinces,<sup>16–18</sup> as well as isolation of *Leptospira* from rodents trapped in the Coastal Province and detection of antibodies against *Leptospira* in cattle, sheep, and donkeys sampled across the country.<sup>19</sup>

Leptospirosis is maintained through chronic renal infection of maintenance hosts, which shed leptospires in their urine. *Leptospira* serovars can infect several host species and different host species can carry several serovars.<sup>3</sup> Rodents are believed to be the most important maintenance hosts for a variety of serovars, but a wide range of mammals, including domestic dogs, cattle, pigs and sheep, can also act as hosts for human pathogenic leptospires.<sup>2</sup>

Globally, leptospirosis is recognized as a re-emerging infection and has been described as a paradigm for an urban health problem emerging as a consequence of the growth of slums.<sup>20</sup> The un-planned nature of slums with their poor sanitation infrastructure often creates conditions that promote the presence of rodents and favors the maintenance of leptospirosis.<sup>20,21</sup> The risk of human leptospirosis infection can vary within slum environments. Studies conducted in Salvador, Brazil indicate that exposure risk clusters at the household level<sup>22</sup> and that rat sightings in and around the household are a risk factor for exposure to leptospires.<sup>20,23</sup>

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As a starting point for advancing our understanding and awareness of leptospirosis in the Kibera settlement in Nairobi, Kenya, we performed a study to determine patterns of *Leptospira* infection in rodent hosts and human-rodent interactions. Specifically, the objectives were to identify the rodent species that were present at this site, detect the presence of pathogenic *Leptospira* spp. in the Kibera rodent population, and describe the nature and frequency of contacts between humans and rodents.

## MATERIALS AND METHODS

**Study setting.** A cross-sectional survey of the rodent population within the Kibera study site was conducted during September–October 2008. The study site lies within the Kibera settlement in Nairobi, Kenya and includes the study area of an ongoing population-based infectious disease surveillance study conducted by the Kenya Medical Research Institute and Centers for Disease Control and Prevention.<sup>13</sup> The site has a human population density of approximately 77,000 persons/km<sup>2</sup><sup>2,13</sup> and is characterized by poor quality housing, poor sanitation, and limited access to clean water.<sup>24</sup>

**Rodent trapping.** The study site was divided into five zones (A–E) on the basis of existing administrative boundaries (Figure 1). A 50 meter × 50 meter trapping area was defined within each zone by using a map of all built structures and aligning one side of the area to an existing straight path or building edge. Approximately every other household within each area was approached for participation in the study. Where possible, traps were set in the enrolled households for all trapping nights in each area. When traps could not be replaced at enrolled households, additional households were enrolled to maintain a target of 50 traps per night. Within each zone, medium-sized (23 cm × 7.5 cm × 9 cm) folding

Sherman live traps (H. B. Sherman Traps Inc., Tallahassee, FL) were placed for a minimum of two and a maximum of six nights to achieve a target of approximately 50 rodents per zone. Traps were baited with dried fish and placed indoors on the floor against walls and under furniture. In most cases, traps were placed in the early afternoon and checked the following morning. Traps that contained rodents were transported to a central processing site and replaced in the same location during the same day. All other traps were re-baited as necessary and replaced.

Trapped rodents were humanely euthanized by halothane (Abbott Laboratories, Abbott Park, IL) overdose.<sup>25</sup> Species identification was made on the basis of morphometric data at the National Museums of Kenya, where all specimens were submitted for archiving (Accession nos. NMK168363–NMK168569 and NMK168633–NMK168664). Whole kidneys were removed by using sterile technique, transferred to sterile cryovials, and kept on ice before storage. Kidney tissues were stored at –80°C and shipped to the University of East London, United Kingdom, in liquid nitrogen.

**Questionnaire survey.** A standardized questionnaire-based survey was conducted to determine the nature and frequency of contacts between rodents and persons in and around households. Questionnaires were administered at 100 households in September–October 2008. In each trapping area, 20 households were selected at random for questionnaire administration from those that had already been recruited for rodent sampling.

**Laboratory diagnostics.** A central portion of approximately 25 mg of each kidney was processed for DNA extraction at the University of East London. Tissues were digested by using 180 µL of buffer ATL (QIAGEN, Hilden Germany) and 20 µL of proteinase K (QIAGEN), mixed and incubated overnight at 56°C, and heated at 80°C for 25 minutes to inactivate any pathogens present in the samples before DNA

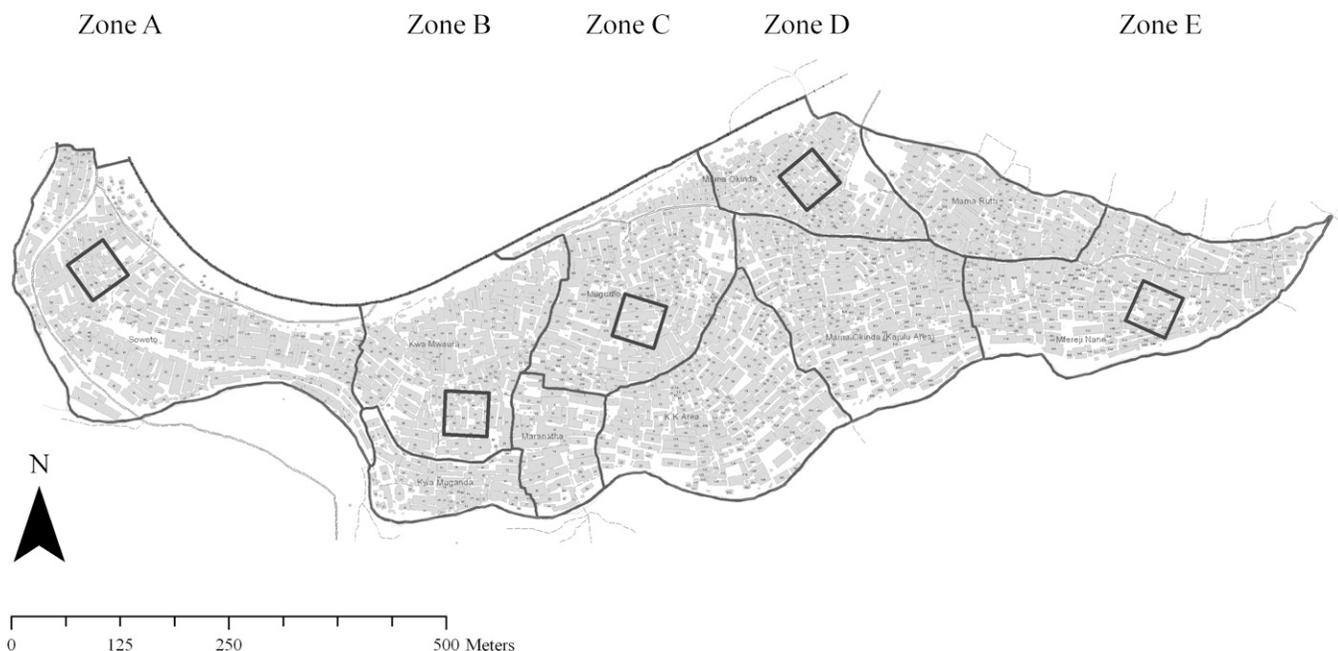


FIGURE 1. Map of the Kibera study site. Small gray filled polygons indicate individual buildings/structures within the study site. Thick gray lines and writing indicate pre-existing administrative boundaries within the site, which were used to define the five study zones (labeled at the top of the figure). The gray squares within each zone illustrate the location of the rodent trapping grids. The scale and orientation of the figure are indicated (bottom left).

extraction. All tissues were processed for DNA extraction by using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol for Purification of Total DNA from Animal Tissues (Spin-Column Protocol) and a final elution volume of 200  $\mu$ L.<sup>26</sup> DNA extracts were shipped to the KIT Biomedical Research Laboratories (Amsterdam, The Netherlands) for further testing.

All kidney DNA extracts were diluted 1:5 in sterile water and tested in triplicate by using the SYBR Green *secY* real-time quantitative polymerase chain reaction (qPCR) protocol for detection of pathogenic *Leptospira*,<sup>27</sup> including melting curve analysis to check the specificity of the amplicons. Samples were classified as positive if two or more test runs yielded an amplification curve with a cycle threshold  $\leq$  38 and a  $T_m$  value between 79.0 and 84.5 °C inclusive. Samples for which one of three initial reactions was positive were retested in triplicate and classified as positive if the repeat run resulted in at least one positive reaction. Two approaches were used to characterize the *Leptospira*-positive samples. Twenty-eight samples were tested by using an adapted Taqman real-time PCR that separates *Leptospira* species into two clades using clade-specific probes targeting the *secY* gene.<sup>28,29</sup> *Leptospira*-positive samples were also submitted for sequencing of the products after using the SYBR Green *secY* qPCR.<sup>28</sup>

**Statistical analyses.** Generalized linear models were used to identify variables associated with the infection status of trapped rodents. In all models, the response variable was a binary measure of infection status (positive or negative) based on the results of the qPCR analyses of kidney samples. The covariates considered were rodent genus (*Rattus* or *Mus*), trapping zone (A–E) and sex (male or female). All statistical analyses were conducted in R.<sup>30</sup>

The phylogenetic analysis included 80 sequences (Table 1 and Figure 2) and was conducted by using MEGA5.<sup>31</sup> The phylogenetic tree was drawn using the neighbor-joining method.<sup>32</sup> Evolutionary distances were computed using the maximum composite likelihood method<sup>33</sup> with distances expressed as the number of basepair substitutions per site.

**Ethics statement.** Written informed consent for sampling and questionnaire administration was obtained from representatives of the study households. The protocols and consent forms were reviewed and approved by the Animal Care and Use and Ethical Review Boards of the Kenya Medical Research Institute (#1191) according to institutional guidelines. The study protocols were also approved by the Institutional Animal Care and Use Committee and Institutional Review Board of the U.S. Centers for Disease Control and Prevention (#5410) and complied with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

## RESULTS

**Rodent trapping.** A total of 237 rodents were trapped from 948 trap placements in 270 Kibera households, with an overall trap success of 24.9% (95% confidence interval [CI] = 22.2–27.8%). Most trapped rodents were *Mus musculus* (n = 194), followed by *Rattus rattus* (n = 33) and *R. norvegicus* (n = 10). Data on the sex of trapped rodents was recorded for 194 rodents, 108 of which were female and 86 of which were male. The overall trap success and relative proportion of *Mus* spp. in the trapped population both varied across the five zones of the study site (Table 2).

**Laboratory diagnostics.** Pathogenic leptospires were detected in the kidneys of 18.3% (41 of 224; 95% CI = 13.5–24.0%) of rodents by qPCR. Only 4 (10.3%) of 39 *Rattus* tested (95% CI = 2.9–24.2%) were positive (n = 3 *R. rattus* and n = 1 *R. norvegicus*) compared with 37 (20.0%) of 185 *M. musculus* (95% CI = 14.5–26.5%). The kidneys of 15 (17.9%) of 84 (95% CI = 10.4–27.7%) male rodents and 14 (13.9%) of 101 (95% CI = 7.8–22.2%) female rodents were positive for pathogenic leptospires by qPCR. Successful amplification of six *Leptospira*-positive samples was achieved by using the Taqman real-time PCR targeting the *secY* gene. All six samples were collected from *M. musculus* rodents and all were classified into clade 1, which includes *L. kirschneri*, *L. interrogans*, *L. noguchii*, and pathogenic strains of *L. meyeri*.<sup>28,29</sup> Sequence data were obtained for three samples, two of which were classified as clade 1 by the Taqman PCR. Phylogenetic analysis indicated that two of these sequences are *L. interrogans*, and one was identified as *L. kirschneri* (Table 1). The nucleotide sequences generated in this study were deposited in GenBank under accession numbers (ARK25 = KF431859, ARK59 = KF431860, and ARK214 = KF431858).

**Questionnaire survey.** One hundred questionnaires were completed and most respondents reported daily sightings of rodents and their excreta in and around Kibera households (Table 3). Missing values (including responses of don't know) were excluded from proportion calculations. Sixty percent of respondents reported seeing groups of five or more rodents in their house on a daily basis, and 66% of respondents reported weekly or daily sightings of fresh excreta in the house (Table 3).

**Statistical analysis.** The generalized linear model of infection status revealed that rodents of the genus *Mus* were significantly more likely to be positive for pathogenic leptospires than rodents of the genus *Rattus* (odds ratio = 15.03, 95% CI = 2.61–86.62) (Table 4). There was also an association between trapping zone and rodent qPCR status, with rodents trapped towards the east of the site (Zones D and E) more likely to be qPCR positive (Table 4). There was no indication of an association between rodent sex and infection status.

## DISCUSSION

Pathogenic leptospires were identified in the Kibera rodent population. Nearly one in five rodents trapped within houses in Kibera had pathogenic leptospires in their kidneys and were thus potentially capable of shedding leptospires into the environment. The questionnaire data showed sightings of multiple rodents and their excreta within households on a daily basis by most study respondents. Previous studies at other urban slum sites have identified rodent sightings around the household as a risk factor for human *Leptospira* exposure.<sup>20,23</sup> As humans acquire leptospirosis through direct or indirect contact with the urine of an infected host, these data, together indicate that the risk of human exposure to pathogenic *Leptospira* spp., is likely to be substantial in Kibera.

According to the results obtained with the Taqman PCR, all positive samples belonged to clade 1, consisting of *L. interrogans*, *L. kirschneri*, *L. noguchii*, and *L. meyeri*. Pathogenic strains of *L. meyeri* and *L. noguchii* are predominantly confined to the Americas and have not been demonstrated in Africa and/or are of minor importance for the epidemiology

TABLE 1  
*Leptospira* strains and samples used in the *secY* sequence-based phylogeny\*

Code	Serovar	Serogroup	Strain	Species
Bif.SE02	Patoc	Semarang	Patoc I	<i>L. biflexa</i>
Bor.BM02	Ballum	Ballum	S102	<i>L. borgpetersenii</i>
Bor.HB06	Jules	Hebdomadis	Jules	<i>L. borgpetersenii</i>
Bor.HB10	Mini	Mini	Sari	<i>L. borgpetersenii</i>
Bor.HB22	Balkanica	Sejroe	1627 Burgas	<i>L. borgpetersenii</i>
Bor.HB23	Polonica	Sejroe	493 Poland	<i>L. borgpetersenii</i>
Bor.HB30	Nyanza	Sejroe	Kibos	<i>L. borgpetersenii</i>
Bor.HB35	Nero	Sejroe	Gamsulin	<i>L. borgpetersenii</i>
Bor.JV03	Poi	Javanica	Poi	<i>L. borgpetersenii</i>
Bor.JV04	Sorexjalna	Javanica	Sorex Jalna	<i>L. borgpetersenii</i>
Bor.PY13	Kwale	Pyrogenes	Julu	<i>L. borgpetersenii</i>
Bor.TA01	Tarassovi	Tarassovi	Perepelitsin	<i>L. borgpetersenii</i>
Bor.TA11	Tunis	Tarassovi	P 2/65	<i>L. borgpetersenii</i>
Bor.TA20	Dikkeni	Sejroe	Mannuthi	<i>L. borgpetersenii</i>
Bor.TA21	Moldaviae	Bataviae	114-2	<i>L. borgpetersenii</i>
Int.AS06	Fugis	Australis	Fudge	<i>L. interrogans</i>
Int.AS07	Bangkok	Australis	Bangkok D-92	<i>L. interrogans</i>
Int.AT08	Sentot	Djasiman	Sentot	<i>L. interrogans</i>
Int.AT11	Djasiman	Autumnalis	Djasiman	<i>L. interrogans</i>
Int.BT02	Bataviae	Bataviae	Van Tienen	<i>L. interrogans</i>
Int.BT03	Paidjan	Bataviae	Paidjan	<i>L. interrogans</i>
Int.CA01	Canicola	Canicola	Hond Utrecht IV	<i>L. interrogans</i>
Int.CA05	Jonsis	Canicola	Jones	<i>L. interrogans</i>
Int.CA07	Broomi	Canicola	Patane	<i>L. interrogans</i>
Int.CA12	Portlandvere	Canicola	My 1039	<i>L. interrogans</i>
Int.CA13	Kuwait	Canicola	136/2/2	<i>L. interrogans</i>
Int.HB01	Hebdomadis	Hebdomadis	Hebdomadis	<i>L. interrogans</i>
Int.HB18	Medanensis	Sejroe	Hond HC	<i>L. interrogans</i>
Int.HB19	Wolffi	Sejroe	3705	<i>L. interrogans</i>
Int.IC01	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	<i>L. interrogans</i>
Int.IC03	Mankarso	Icterohaemorrhagiae	Mankarso	<i>L. interrogans</i>
Int.IC04	Naam	Icterohaemorrhagiae	Naam	<i>L. interrogans</i>
Int.Lai	Lai	Icterohaemorrhagiae	Lai	<i>L. interrogans</i>
Int.PO01	Pomona	Pomona	Pomona	<i>L. interrogans</i>
Int.PO03	Monjakov	Pomona	Monjakov	<i>L. interrogans</i>
Int.PO06	Kennewicki	Pomona	LT 1026	<i>L. interrogans</i>
Int.PY04	Biggis	Pyrogenes	Biggs	<i>L. interrogans</i>
Int.PY09	Manilae	Pyrogenes	LT 398	<i>L. interrogans</i>
Int.PY14	Camlo	Pyrogenes	LT 64-67	<i>L. interrogans</i>
Kir.AT07	Bulgarica	Autumnalis	Nicolaevo	<i>L. kirschneri</i>
Kir.AT19	Butembo	Autumnalis	Butembo	<i>L. kirschneri</i>
Kir.AT20	Bim	Autumnalis	1051	<i>L. kirschneri</i>
Kir.CA02	Galtoni	Canicola	LT 1014	<i>L. kirschneri</i>
Kir.CA03	Bafani	Canicola	Bafani	<i>L. kirschneri</i>
Kir.CA04	Kamituga	Canicola	Kamituga	<i>L. kirschneri</i>
Kir.CY01	Cynopteri	Cynopteri	3522 C	<i>L. kirschneri</i>
Kir.GR03	Ratnapura	Grippotyphosa	Wumalasena	<i>L. kirschneri</i>
Kir.GR04	Vanderhoedeni	Grippotyphosa	Kipod 179	<i>L. kirschneri</i>
Kir.HB03	Kambale	Hebdomadis	Kambale	<i>L. kirschneri</i>
Kir.HB09	Kabura	Hebdomadis	Kabura	<i>L. kirschneri</i>
Kir.IC09	Ndahambukuje	Icterohaemorrhagiae	Ndahambukuje	<i>L. kirschneri</i>
Kir.IC16	Bogvere	Icterohaemorrhagiae	LT 60-69	<i>L. kirschneri</i>
Kir.PO04	Mozdok	Pomona	5621	<i>L. kirschneri</i>
Kir.PO07	Tsaratsovo	Pomona	B 81/7	<i>L. kirschneri</i>
Kir.PO08	Kunming	Pomona	K 5	<i>L. kirschneri</i>
Nog.AT18	Huallaga	Djasiman	M 7	<i>L. noguchii</i>
Nog.BT09	Argentiniensis	Bataviae	Peludo	<i>L. noguchii</i>
Nog.TA18	Carimagua	Shermani	9160	<i>L. noguchii</i>
San.BT05	Kobbe	Bataviae	CZ 320	<i>L. santarosai</i>
San.BT06	Balboa	Bataviae	735 U	<i>L. santarosai</i>
San.HB07	Maru	Hebdomadis	CZ 285	<i>L. santarosai</i>
San.BT08	Brasiliensis	Bataviae	An 776	<i>L. santarosai</i>
San.HB33	Guaricura	Sejroe	Bov. G	<i>L. santarosai</i>
San.HB34	Goiano	Hebdomadis	Bovino 131	<i>L. santarosai</i>
San.IC12	Weaveri	Sarmin	CZ 390	<i>L. santarosai</i>
San.PO05	Tropica	Pomona	CZ 299	<i>L. santarosai</i>
San.PY06	Bagua	Pyrogenes	MW-12	<i>L. santarosai</i>
San.PY07	Alexi	Pyrogenes	HS-616	<i>L. santarosai</i>
San.PY11	Sanmartini	Pyrogenes	CT 63	<i>L. santarosai</i>
San.PY12	Princetown	Pyrogenes	TRVL 112499	<i>L. santarosai</i>

(Continued)

TABLE 1  
Continued

Code	Serovar	Serogroup	Strain	Species
San.TA02	Bakeri	Tarassovi	LT 79	<i>L. santarosai</i>
San.TA03	Atlantae	Tarassovi	LT 81	<i>L. santarosai</i>
San.TA07	Gatuni	Tarassovi	1473 K	<i>L. santarosai</i>
San.TA08	Atchafalaya	Tarassovi	LSU 1013	<i>L. santarosai</i>
San.TA10	Rama	Tarassovi	316	<i>L. santarosai</i>
Wei.JV05	Coxi	Javanica	Cox	<i>L. weilii</i>
Wei.TA13	Langati	Tarassovi	M39039	<i>L. weilii</i>
ARK25	Undefined	Undefined	–	<i>L. interrogans</i>
ARK59	Undefined	Undefined	–	<i>L. kirschneri</i>
ARK214	Undefined	Undefined	–	<i>L. interrogans</i>

\*Samples with code starting ARK are described in this report. Details of the other strains included in this analysis have been reported.<sup>28</sup>

of leptospirosis. Thus, for samples from Africa, placement within clade 1 will support classification as either *L. interrogans* or *L. kirschneri*. Consistent with these results, phylogenetic analysis showed that strains belonging to the species

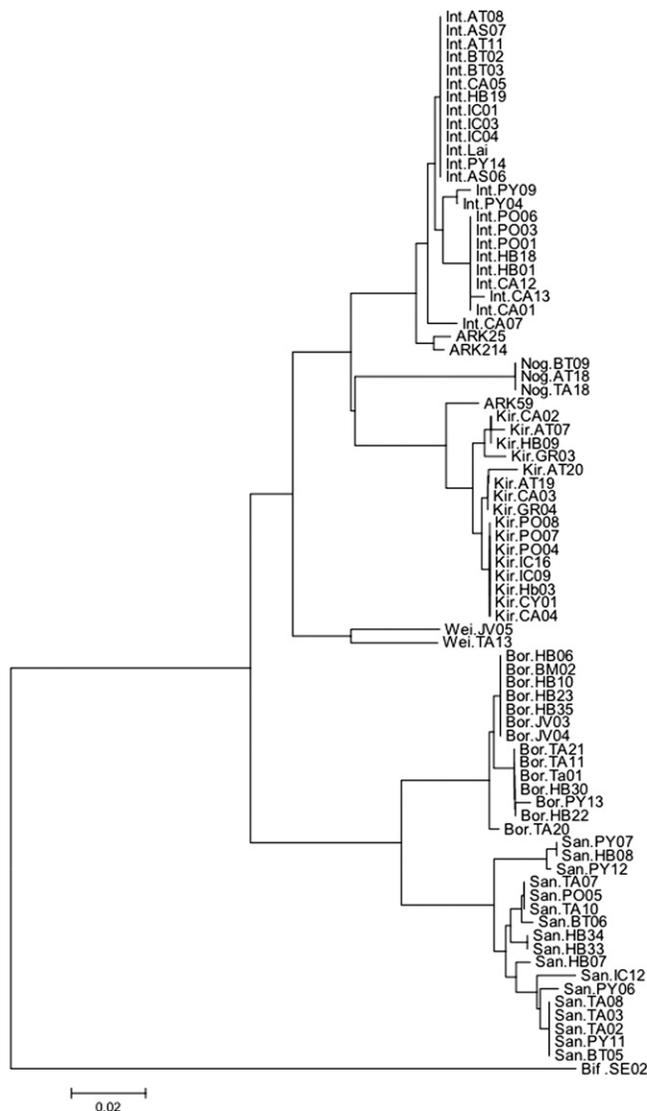


FIGURE 2. Phylogenetic tree of partial *secY* sequences from Kibera samples and *Leptospira* reference strains. Branch lengths are shown in units of number of substitutions per site. Details of the sequences and strains included are given in Table 1. Scale bar indicates nucleotide substitutions per site.

*L. interrogans* and *L. kirschneri* are carried by the Kibera *Mus* population. Phylogenetic speciation of *Leptospira* based on analysis of the *secY* gene used in this study has been described and is highly discriminatory.<sup>27,28,34</sup> Confirmation of the range of pathogenic *Leptospira* maintained in Kibera animal populations will be an important area for future research.

The probability of *Leptospira* infection was significantly higher in *Mus* trapped in Kibera than in *Rattus*. Most previous studies of urban leptospirosis have implicated rodents, and specifically *Rattus* spp., as the maintenance reservoirs for human pathogenic *Leptospira* spp.<sup>23,35–37</sup> The use of Sherman traps may provide one explanation for the relatively low prevalence in the *Rattus* population sampled in this study. These traps may select for smaller (and younger) *Rattus* rodents, in which the *Leptospira* infection prevalence in kidneys may be lower than in adults.<sup>38–40</sup> The mass of the *R. norvegicus* and *R. rattus* rodents trapped in this study ranged from 25 to 194 grams and from 12.5 to 122 grams, respectively, indicating that all of the *Rattus* rodents trapped were juveniles.<sup>39,40</sup> The prevalence of infection detected in *Rattus* rodents in this study may therefore be an underestimate of the prevalence in the *Rattus* population as a whole. Interestingly, the qPCR data showed a high infection prevalence in rodents of the genus *Mus*, which may be important hosts in the epidemiology of leptospirosis in Kibera. This finding highlights the complex multi-host epidemiology of leptospirosis and the importance of considering the role of rats, mice, and other animal hosts in the maintenance and transmission of infection when evaluating human risks.

There is a large population of domestic dogs in Kibera and their density within this study site has been estimated at

TABLE 2

Rodent trapping summary indicating the trapping effort, species trapped, and trap success in each trapping zone within the Kibera study site

Trapping zone	No. trap attempts	No. (%) rodents caught*		Trap success† (%)
		<i>Rattus</i> spp.	<i>Mus</i> spp.	
A	152 nights 30 days	4 0	71 (94.7) 7 (100)	49.3 23.3
B	98 nights	6	40 (87.0)	46.9
C	225 nights 44 days	18 0	17 (48.6) 2 (100)	15.6 4.5
D	149 nights	2	37 (94.9)	25.5
E	250 nights	13	20 (60.6)	13.2
Total	948	43	194 (81.9)	24.9

\*Number of rodents trapped include all individuals, including one case (*Mus* spp. in zone D) in which two rodents were trapped during the same trap attempt.

†Trap success calculations are based on the number of successful trap attempts rather than the number of rodents trapped.

TABLE 3

Household questionnaire data summary indicating the location, type and frequency of different rodent sighting measures within the Kibera study households

Rodent sightings		Frequency (%)			
Location	Type	Daily	Weekly	Monthly	Never
In house	1–4 rodents	69	16	2	13
	≥ 5 rodents	60	11	1	28
	rodent excreta	53	13	0	34
Around/outside the house	1–4 rodents	70	8	5	16
	≥ 5 rodents	57	9	4	30
	rodent excreta	48	11	2	39

740–1,086 dogs/km<sup>2</sup>.<sup>41</sup> The capacity for dogs to act as reservoir hosts for leptospirosis is well described in other settings,<sup>2</sup> and the possible role of dogs and other potential reservoirs in the epidemiology of urban leptospirosis should not be discounted.<sup>42</sup> Our study focused on rodent presence and sightings in and around the household environment, which have been identified as risk factors for human exposure risks in slum settings. Previous studies in urban areas have also identified contaminated surface waters as a source of human exposure risk<sup>36,43</sup> and household flooding risk and proximity to sewers as predictors of human exposure risk.<sup>20</sup> The routes of indirect exposure of humans to leptospires shed into environmental water sources by animal hosts should also be considered in future studies to evaluate human disease risks.

The multivariate model indicates that rodents trapped in zones D and E, to the east of the site, were significantly more likely to be positive than rodents trapped in the reference zone A (Figure 1). The total area of the population-based infectious disease surveillance study site, within which these five trapping areas were established, is just 0.38 km<sup>2</sup>.<sup>2,13</sup> and these data indicate substantial variation in the prevalence of leptospirosis in rodents, even over the relatively short distances. This zone effect was seen when the rodent genus variable was also included in the model, indicating that this effect is not entirely attributable to differences in the distribution of these different hosts between zones. Within the Kibera site, the terrain slopes down towards a river, which runs along the southern boundary of the site, and much of the eastern part of the site is at lower elevation than the western part. Fine-scale spatial variation in human exposure risk has been demonstrated at other slum sites<sup>20,22</sup> and geographic variables, such as reduced elevation, flooding risk, proximity to drainage, and proximity to rubbish dumps, have been described as risk factors for human exposure to *Leptospira* spp.<sup>20,23</sup> The influence of geographic variables upon animal and human infection risk at

TABLE 4

Results of multivariate generalized linear model analysis of risk factors for positive rodent quantitative polymerase chain reaction status within the trapped Kibera rodent population\*

Variable	Level	OR	95% CI	No.	P
Intercept	–	–	–		1.63 × 10 <sup>-7</sup>
Trapping zone	A	–	–	82	–
	B	0.94	0.23–4.08	46	0.96
	C	3.02	0.76–12.03	37	0.12
	D	4.92	1.65–14.63	39	0.004
	E	98.58	18.03–539.03	33	1.18 × 10 <sup>-7</sup>
Genus	<i>Rattus</i>	–	–	43	–
	<i>Mus</i>	15.03	2.61–86.62	194	0.002

\*OR = odds ratio, CI = confidence interval.

the Kibera site and the spatial correspondence between rodent prevalence and human risk warrant further investigation.

This study had some limitations. The kidney extracts were diluted to reduce the influence of qPCR inhibitors present in these kidney tissue samples. This dilution step, as well as the effect of any residual inhibition in tissue extracts, could result in false-negative qPCR results. Therefore, 18.3% is considered to be a conservative estimate of true prevalence in the Kibera rodent population. Renal colonization as high as 80.3% has been detected by culture (83.9% by PCR) among rats sampled in an urban setting in Salvador, Brazil.<sup>39</sup> Other studies have reported carriage rates of 35.3% in urban rodent populations in Madagascar through combined use of molecular, serologic, and culture approaches;<sup>44</sup> 20% in urban *R. norvegicus* trapped in Colombia by culture;<sup>37</sup> 21.7% *R. norvegicus* trapped in an urban slum in Peru by PCR;<sup>36</sup> and 20% of rodents trapped near urban areas in Peru by PCR.<sup>45</sup> Culture and isolation of *Leptospira* strains were beyond the scope of this study but are essential to enable isolate typing and are a priority for future research at this site. Characterization of *Leptospira* serovars present in animal populations in Kibera may help to shed light on the relative importance of different maintenance hosts in this setting, as well as contributing to the development and validation of locally appropriate diagnostic tests for clinical use.

In this study, we have demonstrated the presence of pathogenic leptospires in the Kibera rodent population. Although the epidemiology of leptospirosis is complex and humans can acquire infection through indirect or direct contacts with a variety of animal reservoir hosts, rodents are frequently identified as important reservoirs in urban slum settings particularly, and household rodent sighting frequencies have been shown to correlate with human exposure risks in other urban slum settings. Our findings, which are consistent with reports from slum sites in South America<sup>20</sup> and Asia,<sup>11</sup> indicate that human exposure to pathogenic *Leptospira* spp. may be considerable in Kibera. These data contribute to the growing body of evidence suggesting that leptospirosis may be an under-recognized but important cause of human illness, specifically in urban slum populations.

Despite increasing recognition of the clinical threat posed by leptospirosis, the impact of leptospirosis on the health and productivity of animal and human populations continues to be widely unrecognized and under-reported. In many human populations, including Kibera, the burden of undifferentiated febrile illness is significant.<sup>13</sup> Broader diagnostic testing, including consideration of acute leptospirosis, is needed to determine the causes of these illnesses. Ultimately, these data will be useful to raise awareness of leptospirosis among clinicians and promote appropriate clinical management of cases.

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## REFERENCES

- World Health Organization, 1999. Leptospirosis worldwide, 1999. *Wkly Epidemiol Rec* 74: 237–242.
- Levett PN, 2001. Leptospirosis. *Clin Microbiol Rev* 14: 296–326.
- Hartskeerl RA, Collares-Pereira M, Ellis WA, 2011. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infect* 17: 494–501.
- Biggs HM, Bui DM, Galloway RL, Stoddard RA, Shadomy SV, Morrissey AB, Bartlett JA, Onyango JJ, Maro VP, Kinabo GD, Saganda W, Crump JA, 2011. Leptospirosis among hospitalized febrile patients in northern Tanzania. *Am J Trop Med Hyg* 85: 275–281.
- Levett PN, 2003. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clin Infect Dis* 36: 447–452.
- Machang'u RS, Mgode GF, Assenga J, Mhamphi G, Weetjens B, Cox C, Verhagen R, Sondij S, Goris MG, Hartskeerl RA, 2004. Serological and molecular characterization of *Leptospira* serovar Kenya from captive African giant pouched rats *Cricetomys gambianus* from Morogoro Tanzania. *FEMS Immunol Med Microbiol* 41: 117–121.
- Holt J, Davis S, Leirs H, 2006. A model of leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. *Acta Trop* 99: 218–225.
- Mgode GF, Machang'u RS, Goris MG, Engelbert M, Sondij S, Hartskeerl RA, 2006. New *Leptospira* serovar Sokoine of serogroup Icterohaemorrhagiae from cattle in Tanzania. *Int J Syst Evol Microbiol* 56: 593–597.
- Matthias MA, Ricaldi JN, Cespedes M, Diaz MM, Galloway RL, Saito M, Steigerwalt AG, Patra KP, Ore CV, Gotuzzo E, Gilman RH, Levett PN, Vinetz JM, 2008. Human leptospirosis caused by a new, antigenically unique *Leptospira* associated with a *Rattus* species reservoir in the Peruvian Amazon. *PLoS Negl Trop Dis* 2: e213.
- Reller ME, Bodinayake C, Nagahawatte A, Devasiri V, Kodikara-Arachichi W, Strouse JJ, Flom JE, Dumler JS, Woods CW, 2011. Leptospirosis as frequent cause of acute febrile illness in southern Sri Lanka. *Emerg Infect Dis* 17: 1678–1684.
- Kendall EA, LaRocque RC, Bui DM, Galloway R, Ari MD, Goswami D, Breiman RF, Luby S, Brooks WA, 2010. Leptospirosis as a cause of fever in urban Bangladesh. *Am J Trop Med Hyg* 82: 1127–1130.
- Ismail TF, Wasfy MO, Abdul-Rahman B, Murray CK, Hospenhal DR, Abdel-Fadeel M, Abdel-Maksoud M, Samir A, Hatem ME, Klena J, Pimentel G, El-Sayed N, Hajjeh R, 2006. Retrospective serosurvey of leptospirosis among patients with acute febrile illness and hepatitis in Egypt. *Am J Trop Med Hyg* 75: 1085–1089.
- Feikin DR, Olack B, Bigogo GM, Audi A, Cosmas L, Aura B, Burke H, Njenga MK, Williamson J, Breiman RF, 2011. The burden of common infectious disease syndromes at the clinic and household level from population-based surveillance in rural and urban Kenya. *PLoS ONE* 6: e16085.
- World Health Organization, 2004. *Leptospirosis in Kenya*. Available at: [http://www.who.int/csr/don/2004\\_06\\_17a/en/](http://www.who.int/csr/don/2004_06_17a/en/). Accessed August 26, 2013.
- Ari MD, Guracha A, Fadeel MA, Njuguna C, Njenga MK, Kalani R, Abdi H, Warfu O, Omballa V, Tetteh C, Breiman RF, Pimentel G, Feikin DR, 2011. Challenges of establishing the correct diagnosis of outbreaks of acute febrile illnesses in Africa: the case of a likely *Brucella* outbreak among nomadic pastoralists, northeast Kenya, March–July 2005. *Am J Trop Med Hyg* 85: 909–912.
- de Geus A, Wolff JW, Timmer VE, 1977. Clinical leptospirosis in Kenya (II): a field study in Nyanza Province. *East Afr Med J* 54: 125–132.
- de Geus A, Wolff JW, Timmer VE, 1977. Clinical leptospirosis in Kenya (I): a clinical study in Kwale District, Coast Province. *East Afr Med J* 54: 115–124.
- Forrester AT, Kranendonk O, Turner LH, Wolff JW, Bohlander HJ, 1969. Serological evidence of human leptospirosis in Kenya. *East Afr Med J* 46: 497–506.
- Ball MG, 1966. Animal hosts of leptospires in Kenya and Uganda. *Am J Trop Med Hyg* 15: 523–530.
- Reis RB, Ribeiro GS, Felzemburgh RD, Santana FS, Mohr S, Melendez A, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI, 2008. Impact of environment and social gradient on *Leptospira* infection in urban slums. *PLoS Negl Trop Dis* 2: e228.
- Oliveira DS, Guimarães MJ, Portugal JL, Medeiros Z, 2009. The socio-demographic, environmental and reservoir factors associated with leptospirosis in an urban area of north-eastern Brazil. *Ann Trop Med Parasitol* 103: 149–157.
- Maciel EA, de Carvalho ALF, Nascimento SF, de Matos RB, Gouveia EL, Reis MG, Ko AI, 2008. Household transmission of *Leptospira* infection in urban slum communities. *PLoS Negl Trop Dis* 2: e154.
- Sarkar U, Nascimento SF, Barbosa R, Martins R, Nuevo H, Kalafanos I, Grunstein I, Flannery B, Dias J, Riley LW, Reis MG, Ko AI, 2002. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. *Am J Trop Med Hyg* 66: 605–610.
- Gulis G, Mulumba JA, Juma O, Kakosova B, 2004. Health status of people of slums in Nairobi, Kenya. *Environ Res* 96: 219–227.
- Mills JN, Childs JE, Ksiazek TG, Peters CJ, Velleca WM, 1995. *Methods for Trapping and Sampling Small Mammals for Virologic Testing*. Available at: <http://www.cdc.gov/hantavirus/resources/materials.html>. Accessed August 16, 2013.
- QIAGEN, 2006. *DNeasy Blood and Tissue Handbook*. Available at: <http://www.qiagen.com/Knowledge-and-Support/Resource-Center/Resource-Search/?q=DNeasy%3b&l=en%3b>. Accessed August 26, 2013.
- Ahmed A, Engelberts MF, Boer KR, Ahmed N, Hartskeerl RA, 2009. Development and validation of a real-time PCR for detection of pathogenic *Leptospira* species in clinical materials. *PLoS ONE* 4: e7093.
- Victoria B, Ahmed A, Zuerner RL, Ahmed N, Bulach DM, Quinteiro J, Hartskeerl RA, 2008. Conservation of the S10-spc  $\alpha$  locus within otherwise highly plastic genomes provides phylogenetic insight into the genus *Leptospira*. *PLoS ONE* 3: e2752.
- Houemenou G, Ahmed A, Libois R, Hartskeerl RA, 2013. *Leptospira* spp. prevalence in small mammal populations in Cotonou, Benin. *ISRN Epidemiol* 2013.
- R Development Core Team, 2009. *R: A Language and Environment for Statistical Computing*. Available at: <http://www.R-project.org>. Accessed August 26, 2013.

31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
32. Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425.
33. Tamura K, Nei M, Kumar S, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci USA* 101: 11030–11035.
34. Ahmed N, Devi SM, Valverde Mdl A, Vijayachari P, Machang'u RS, Ellis WA, Hartskeerl RA, 2006. Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. *Ann Clin Microbiol Antimicrob* 5: 28.
35. Ko AI, Reis MG, Dourado CMR, Johnson WD, Riley LW, 1999. Urban epidemic of severe leptospirosis in Brazil. Salvador Leptospirosis Study Group. *Lancet* 354: 820–825.
36. Johnson MA, Smith H, Joseph P, Gilman RH, Bautista CT, Campos KJ, Céspedes M, Klatsky P, Vidal C, Terry H, Calderon MM, Coral C, Cabrera L, Parmar PS, Vinetz JM, 2004. Environmental exposure and leptospirosis, Peru. *Emerg Infect Dis* 10: 1016–1022.
37. Agudelo-Flórez P, Londoño AF, Quiroz VH, Angel JC, Moreno N, Loaiza ET, Muñoz LF, Rodas JD, 2009. Prevalence of *Leptospira* spp. in urban rodents from a groceries trade center of Medellín, Colombia. *Am J Trop Med Hyg* 81: 906–910.
38. Benacer D, Zain SN, Amran F, Galloway RL, Thong KL, 2013. Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* isolates from the urban rat populations of Kuala Lumpur, Malaysia. *Am J Trop Med Hyg* 88: 704–709.
39. de Faria MT, Calderwood MS, Athanzio DA, McBride AJ, Hartskeerl RA, Pereira MM, Ko AI, Reis MG, 2008. Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. *Acta Trop* 108: 1–5.
40. Easterbrook JD, Kaplan JB, Vanasco NB, Reeves WK, Purcell RH, Kosoy MY, Glass GE, Watson J, Klein SL, 2007. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect* 135: 1192–1199.
41. Halliday JEB, 2010. *Animal Sentinel Surveillance: Evaluating Domestic Dogs as Sentinels for Zoonotic Pathogen Surveillance*. Available at: <http://www.era.lib.ed.ac.uk/handle/1842/4794>. Accessed August 26, 2013.
42. Vinetz JM, 2001. Leptospirosis. *Curr Opin Infect Dis* 14: 527–538.
43. Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, Segura ER, Gilman RH, Gotuzzo E, Vinetz JM, 2006. Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira*. *PLoS Med* 3: 1329–1340.
44. Rahelinirina S, Leon A, Hartskeerl RA, Sertour N, Ahmed A, Raharimanana C, Ferquel E, Garnier M, Chartier L, Duplantier JM, Rahalison L, Cornet M, 2010. First isolation and direct evidence for the existence of large small-mammal reservoirs of *Leptospira* sp. in Madagascar. *PLoS ONE* 5: e14111.
45. Bunnell JE, Hice CL, Watts DM, Montrueil V, Tesh RB, Vinetz JM, 2000. Detection of pathogenic *Leptospira* spp. infections among mammals captured in the Peruvian Amazon basin region. *Am J Trop Med Hyg* 63: 255–258.