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Seroprevalence And Risk Factors For Leptospirosis In An Urban Community Of Puerto Rico

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Seroprevalence and Risk Factors for Leptospirosis in an Urban Community of Puerto Rico

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Abstract

Introduction: Leptospirosis is an important cause of morbidity and mortality in Puerto Rico, but the full burden remains unknown due to underreporting and misdiagnosis as dengue fever.

Methods: A cross-sectional study paired with rodent trapping was carried out to determine the prevalence and risk factors for prior *Leptospira* infection in an urban community within San Juan, Puerto Rico. A mixed effect model was built to test for risk factors while controlling for grouping effects across neighborhood blocks.

Results: Among 202 human individuals enrolled, 55 (27.3%, 95% CI: 20.9-33.3%) had *Leptospira* agglutinating antibodies indicative of previous exposure to the bacteria. MLST testing of rodent samples indicated that *L. borgpetersenii* Ballum and *L. interrogans* Icterohaemorrhagiae are circulating among rodents in the study area. *L. borgpetersenii* Ballum was also isolated from the kidney of a rodent and showed agglutination against sera from 16 (7.9%) of humans enrolled, indicating that this serogroup is actively circulating among humans and rodents in this community. Living farther away from the Caño Martin Peña canal was associated with decreased risk of leptospire infection, with each meter of distance from the canal associated with an 0.6% decrease in risk of infection (OR:0.994, 95%CI: 0.990 – 0.998) and reported occurrence of household flooding was associated with lower likelihood of infection with leptospires (OR: 0.12, 95% CI: 0.04 – 0.37).

Conclusion: While leptospirosis is often associated with work-related exposures or densely packed urban slum communities, these results suggest that household-related exposures are an important factor in leptospire transmission in low-income urban non-slum communities. This study was a unique opportunity to identify a strain of a zoonotic disease circulating within both rodents and humans in a community facing a variety of environmental risk factors. This confirmation that leptospires are actively circulating in this community could lead to improved diagnostic awareness among healthcare providers and improved patient outcomes for this treatable disease.

Introduction

Leptospirosis is a zoonotic disease that causes a yearly loss of 2.9 million disability adjusted life years (DALYs).[1] Leptospires thrive in the kidneys of rodents and other animals and are excreted in their urine, often contaminating water, mud, or soil. Infection occurs when leptospires enter the body through abrasions on the skin or through mucosa membranes (eyes, nose and mouth). [2, 3] The result of infection with leptospires can range from asymptomatic infection to a self-limited acute febrile illness to life-threatening disease. An estimated 5–15% of hospitalized leptospirosis patients die due to pulmonary hemorrhage, acute renal failure, multi-organ failure, and septic shock.[2] Worldwide, leptospirosis is estimated to result in approximately 1.03 million cases and 55,000 deaths per year.[1, 2]

Though leptospirosis has been traditionally associated with occupational risk factors such as cleaning sewers, work with animals, or agricultural work, more recently densely packed urban communities have been shown to be important transmission venues for leptospirosis.[4-6] Studies in Latin American countries have found the risk of leptospire infection to be associated with household environmental factors such as residence in flood-risk regions with open sewers and proximity to accumulated refuse, rat sightings, and the presence of chickens. [7]

Although leptospirosis is a reportable disease,[8] the true incidence of the disease in Puerto Rico is unknown due to low clinical awareness, lack of routine diagnostic testing and underreporting.[9, 10] Misdiagnosis of leptospirosis as dengue fever is an ongoing problem in areas of co-endemicity, such as Puerto Rico. From 2000 to 2009, approximately 15–100 suspected cases of leptospirosis were reported to the Puerto Rico

Department of Health (PRDH) each year.[11] A CDC study of a concurrent dengue and leptospirosis epidemic in 2010 identified and confirmed 29 fatal cases and 147 non-fatal cases of leptospirosis across several "hot-spots" within Puerto Rico that had previously been undiagnosed, supporting the conclusion that leptospirosis is underreported on the island.[12] Given the underreporting and possible misdiagnosis of leptospirosis, the true community burden of the disease within Puerto Rico is unknown.

The Caño Martin Peña urban community in San Juan, Puerto Rico surrounds a canal after which the community is named. This canal has become clogged and stagnant due to debris and illegal dumping, leading to unsanitary conditions and flooding at major rainfall events.[13] This potentially contaminated floodwater source, along with known rodent infestation in the area, create conditions that make transmission of bacterial diseases such as leptospirosis highly likely in this community.[13] Though the Caño Martin Peña community is not a slum, it still has numerous factors including a dense population that make it a likely transmission site for leptospirosis. In order to investigate the leptospirosis burden in Puerto Rico, we studied the Caño Martin Peña area to look for evidence of leptospiral infection among humans and carriage among rodents.

Here, we report findings of a seroprevalence survey and rodent trapping survey, which aimed to determine the impact, risk factors, and circulating serogroups of leptospirosis among the urban population in Caño Martin Peña, a community in San Juan, Puerto Rico.

Methods

Study Site and Sampling Methodology

The study was conducted in the Caño Martin Peña neighborhood, a community situated in San Juan, a municipality of 365,575 inhabitants, in Northern Puerto Rico (Figure 2). The Caño Martin Peña neighborhood surrounds the Caño Martin Peña canal, a waterway that should flow between two lagoons but is mostly stagnant due to clogging with trash and debris (Figure 2). The Caño Martin Peña neighborhood is a densely-populated working class settlement. A study site was established which was composed of four census tracts in an area of 1.37 $km^{2.}$ A U.S. Census of the study site in 2010 identified a population of 5213 households and 12,690 inhabitants.

The Caño Martin Peña (CMP) study area was selected for this study because the site was known to contain environmental factors such as rodents, standing water, and flooding which could be conducive to leptospirosis, and a CDC study had recently identified at least 2 cases of leptospirosis in the area.[12] The CMP study area was defined using GIS mapping with 2010 Census Data. Using available maps of the CMP study area, blocks of similar size (about 25 roofs per block) were defined, as delineated by roads, or when necessary, by eye, in order to keep all blocks approximately the same size. All 160 blocks within the study area were systematically assigned a unique number 1 to 160. Using 2010 Census data of the study area (Census tracts 37, 38, 45, 46), we assumed 25 roofs per block and 2 to 4 individuals living under each roof. Using a random number generator, 24 random numbers ranging 1 through 160 were selected. Then, all roofs within each of the randomly selected blocks were numbered, and a random number generator was used to randomly select 30% of the roofs in each selected block. Study teams were provided geocoded maps of the selected blocks with the selected houses labeled using the mobile device application Map PDF (Avenza). Study teams visited each

pre-selected block and house up to 3 times to try to offer enrollment to the residents. Abandoned or demolished homes were recorded as such.

Ethics

Participants were enrolled according to written informed consent procedures approved by the Institutional Review Boards of the Puerto Rico Department of Public Health and Yale School of Public Health.

Head of Household Survey:

A study team composed of community health workers and phlebotomists conducted interviews during house visits to obtain information on socioeconomic indicators, exposures to sources of environmental contamination, and potential reservoirs in and around the household. The head-of-household, defined as the member who self-identified as best able to answer questions about the household, was interviewed to determine total income for the household. Subjects were asked to report the highest number of rats sighted within and around the household property, presence of dogs, cats, chickens, ducks, and horses around the household, and frequency and severity of flooding in and around the household.

Individual Survey:

The study team of community health workers and phlebotomists interviewed each household member individually to obtain information on demographic and socioeconomic indicators, employment and occupation, use of personal protective equipment, and exposures to sources of environmental contamination and potential reservoirs in the household and workplace.

Visual Inspection:

The study team surveyed the area immediately surrounding the household property for signs of rodent infestation using an adapted version of a standardized form checking for open sewers, plant debris, and animal food, standing water, rodent burrows, and refuse deposits.[14] GIS Coordinates of each household were collected and recorded during the household visit using Map PDF (Avenza) on mobile devices.

Rodent Trapping:

Rodent species within CMP were targeted to test for pathogenic *Leptospira* serovars. Fieldwork consisted of standard trapping techniques with medium-sized tomahawk live traps baited with tuna fish and small Sherman live traps baited with rolled oats for three consecutive nights. For the first night of trapping, the study team set one tomahawk and two Sherman traps in the yards of six willing study participants. For the subsequent two nights, the study team placed traps in communal trash areas and in a riparian zone adjacent to homes in CMP. Traps were set at dusk in lines of 40 (80 traps total) and checked at dawn. Captured individuals were held in the traps until euthanized and processed at a local veterinary office in San Juan.

Rodent species were distinguished arbitrarily using morphological characteristics in body shape and tail to body length ratios. Each animal collected was assigned a unique field number to associate samples with the corresponding geographical location, as collected with a Garmin unit. Euthanized animals were first anesthetized with isoflurane. After the animal was deeply anesthetized or dead from anesthetic overdose, cervical dislocation was performed. Urine, blood (serum and clot), a kidney fragment, and a tail clipping were collected aseptically at necropsy. Urine was immediately placed in a

culture tube and stored at room temperature. In some animals (n = 6) there was not enough urine to collect a sample, in which case the bladder was collected. Kidney and blood samples were immediately stored at -80°C until analysis.

Rodent Sampling:

Isolation of leptospires from urine, DNA extraction and qPCR

Urine samples or bladder tissue were inoculated immediately following collection into tubes containing 5ml of EMJH medium and incubated at room temperature. Cultures were checked three times a week under dark-field microscopy for the presence of leptospires for up to 28 days. When leptospires were observed, 1 ml of the culture was filtered through 0.45 μm filters, transferred to fresh EMJH medium and incubated at 29^{O}C. For DNA extraction, 10 ml of late-log cultures were harvested by centrifugation (8,000 *g*, for 10 min) and extracted using the Maxwell 16 Cell DNA Purification Kit (Promega). For kidney specimens, DNA was prepared from 25mg of previously frozen tissue, with the Maxwell 16 Tissue DNA Purification Kit (Promega).

The concentration of pathogenic leptospires in the rodent specimens was assessed using a qPCR targeting the *lipL32* gene [15] with some modifications. [16] An additional qPCR targeting a housekeeping gene in rodents (*gadph*) was used to monitor for PCR inhibition.[17]

Identification, MLST, serogrouping and virulence testing

To identify the circulating *Leptospira* species, a fragment of the *secY* gene was amplified and sequenced from all qPCR-positive samples and urine isolates using a protocol described elsewhere with some modifications.[16] In addition, a multi locus sequence typing (MLST) scheme for 7 loci was applied to the urine isolate and a

selection of kidney extracts using a previously described method with some modifications [18] The allelic profiles obtained were used to assign sequence types (STs) to all samples using the *Leptospira* MLST website. [19] To determine the serogroup of the urine isolate, a microscopic agglutination test was conducted with 20 rabbit antisera for the major *Leptospira* serogroups.

Virulence testing

To determine if the urine isolate was virulent, two 22-day-old Golden Syrian male hamsters were infected at a dose of 10^8 leptospires via intraperitoneal route.

Serological Analysis:

Sera were processed from blood samples collected from subjects during house visits and from rodents after trapping. The microscopic agglutination test (MAT) was performed to evaluate for serologic evidence of a prior *Leptospira* exposure. A panel of 28 reference strains were used which included *L. interrogans* serogroup Icterohaemorrhagiae and *L. borgspetersenii* serogroup Ballum (Appendix 1). Additionally, we included in the panel the rodent isolate obtained from the urine samples. Screening was performed with serum dilutions of 1：50 and 1：100. Agglutination against a serum dilution at concentration of 1:50 or higher was considered a seropositive result. When agglutination was observed at a dilution of 1：100, the sample was titrated to determine its highest titer. The presumptive serogroup was defined as the serogroup for which the sample had the highest titer. In cases in which the sample had the same highest titer for several serogroups, the final serogroup result was defined as mixed.

Statistical Methods:

Information was collected on paper forms and subsequently double-entered into a Filemaker database. Statistical analyses were conducted using SAS (Studio) and a p-value <0.05 in two sided testing was used as the criterion for a significant difference. A titer greater or equal to 1:50 was used to define the presence of *Leptospira* antibodies in the analyses. In order to compare the average *Leptospira* concentration in the kidney of each rodent species, a one-way ANOVA with Tukey correction was conducted.

Crude prevalence of prior leptospirosis infection was calculated along with a 95% confidence interval. Prevalence was also stratified by age and gender. We used Student's t-test for continuous variables. A generalized linear mixed model with a Poisson distribution was created to identify the individual effect of factors on risk of infection and to account for clustering within blocks. Variables that were significant in univariate study were included in the multivariate regression. A model was fit in Glimmix using random effects for block grouping and including home flooding and distance from canal as covariates, controlling for age and sex.

ArcGIS software (10.2) was used to plot human households and rodent trapping sites and to create maps coded for seropositive or seronegative results. Distance of each sampled household from the Caño Martin Peña canal was calculated using the ArcGIS spatial analysis tools package. Clustering analysis was not conducted because the underlying assumption of spatial randomness does not hold.

Results

Seroprevalence Survey:

Among 11,963 eligible residents from the community site, 202 (1.7%) were enrolled in the study. Compared to eligible residents who did not participate in the study,

study subjects had a higher proportion of females (60.1% of enrolled subjects versus 42.9% of unenrolled subjects, respectively; $P<0.05$) and older mean age (48.1±21.6 versus 39.7±25.0 years, respectively; $P<0.05$). Median annual household income for study subjects was US$ 15,000. Among the subjects, 57% had not graduated from high school. Among subjects ≥18 years of age, 39.9% were employed.

Among the 202 subjects, 55 (27.3%) had *Leptospira* agglutinating antibodies, as determined by the presence of MAT titer ≥1:50. Highest titers were directed against *L. interrogans* serogroup Icterohaemorrhagiae in 12 (6.0%) of the 202 subjects with *Leptospira* antibodies, against serogroup Autumnalis in 6 (3.0%) of subjects and against the CMP isolate *L. borgpetersenii* serogroup Ballum in 4 (2.0%) of subjects. (Table 1) Icterohaemorrhagiae was the predominant serogroup recognized for the range of highest reciprocal titers. Among seropositive subjects, 19 (35.8%) had mixed titers in which highest reactions occurred against more than one serogroup. Among those 19 subjects with mixed serogroup, 13 (68.4%) showed reactivity against Icterohaemorrhagiae and 5 (26.3%) showed reactivity against serogroup Ballum. Overall, 7.9% of subjects had antibodies against the serogroup Ballum strain isolated from a rodent in CMP.

Prevalence was highest among adolescents and young adults (38.1% and 44.4% for age groups 15–24 and 35-44 years, respectively). (Figure 1) Prevalence was also high in children, with 31.6% of children 5–14 years of age showing evidence for a prior exposure to *Leptospira*. The prevalence was slightly higher in males than females (32.5% versus 23.0%, respectively; $P=0.2095$) (Table 2).

Rodent analysis:

A total of 18 rodents (5 *Mus musculus*, 10 *Rattus norvegicus*, and 3 *Rattus rattus*) were captured in Caño Martín Peña neighborhood. Of the 18 animals captured, 11 were qPCR positive for pathogenic *Leptospira*. Specifically, all 5 *M. musculus* captured were positive, but only 2 of 3 *R. norvegicus* and 4 of 10 *R. rattus* were (Table 3). The concentration of *Leptospira* in positive kidneys in *M. musculus* was 2.17×10^8 GC/g, significantly higher than the concentrations found in *R. rattus* and *R. norvegicus* (2.04×10^4 and 3.77×10^4 GC/g, respectively) ($p=0.0027$) (Table 3).

In order to identify the *Leptospira* species colonizing the kidneys of the captured rodents, we sequenced a fragment of the *secY* gene of all qPCR-positive samples. All *M. musculus*, one *R. norvegicus* and one *R. rattus* sequences showed 99% identity to *L. borgpetersenii* sequences in GenBank. In addition, 2 *R. rattus* sequences were 100% identical to *L. interrogans secY* sequences (Table 3). Subsequently, we performed a MLST to 4 animals containing *L. borgpetersenii* and 2 animals containing *L. interrogans*. Mice M2 and M3 resulted in sequence type 149, which is shared by isolates identified as *L. borgpetersenii* serogroup Ballum in the Leptospira MLST website. In animals N1 and R2, we could amplify only 6 and 4 of the genes required for the MLST procedure, respectively. Nonetheless, the sequence type that matched the partial profiles obtained was 149, the same as that of animals M2 and M3. Finally, the MLST for animal R3 was unsuccessful, but animal R4 presented a partial profile that matched sequence type 17, which is shared by isolates identified as *L. interrogans* serogroup Icterohaemorrhagiae. Altogether these results suggest that at least two pathogenic *Leptospira* species, *L. interrogans* Icterohaemorrhagiae and *L. borgpetersenii* Ballum, are circulating among this rodent population, with the latter being present in the three rodent species captured.

Urine isolate:

We obtained a single urine isolate from *M. musculus* M2 which was identified as *L. borgpetersenii* serogroup Ballum based on the secY sequence (99% identity to *L. borgpetersenii* sequences in GenBank), MLST sequence type (149, which is shared by *L. borgpetersenii* Ballum isolates), and serogrouping (maximum titer against antibodies from serogroup Ballum). Interestingly, the *secY* sequence and all the MLST genes were identical to the ones from their corresponding kidney specimen. Finally, we tested the virulence of the M2 isolate in a hamster model of infection and found that a dose of 10^8 cells via intraperitoneal route resulted in hamster death within 5 days, confirming that the isolate M2 was a virulent strain.

Risk Factors:

Living in a home closer to the canal was associated with increased risk of infection, both independently and after controlling for block effects, age, sex, and reported frequency of household flooding. Living farther away from the Caño Martin Peña canal was associated with decreased risk of leptospire infection, with each meter of distance from the canal associated with an 0.6% decrease in risk of infection (OR:0.994, 95%CI: 0.990 – 0.998)

Those who lived in a household that reported seldom, sometimes, often, or always flooding in the rain were 80% less likely to be exposed to leptospires, as compared to living in a household that never flooded (OR: 0.20, 95% CI: 0.06-0.62). After adjusting for distance from the canal, block grouping, age, and sex, those who reported flooding in their homes were still shown to be protected against exposure to leptospires (OR: 0.12, 95% CI: 0.04- 0.37). (Table 4)

There was some clustering of risk by block location. In a generalized linear mixed model, people living in one block out of the 24 blocks sampled were significantly more likely to be seropositive than average (P = 0.0158).

Discussion

We conducted a serosurvey and animal trapping in an urban community in San Juan, Puerto Rico to determine the levels of exposure to pathogenic *Leptospira* and to identify the circulating serovars. This significant problem affecting this poor urban population was previously unstudied and unrecognized. We determined that high proportions of Caño Martin Peña inhabitants of all sexes and age groups are exposed to pathogenic leptospires and that exposure is likely widespread and occurring in and around the household environment. The rodent trapping and sampling revealed that a high proportion of rodents are carriers of pathogenic leptospires serogroups and that these serogroups are also found in the human population. The highest titers in rodents were against the same serogroups as found in the humans, indicating that rodents are reservoirs of high transmission of this disease. Proximity of residence to the canal was associated with increased risk of exposure to leptospires, indicating that there are significant structural factors in this area that are linked to transmission.

Leptospirosis is a widespread problem in the Caño Martin Peña community, infecting children, adults, and the elderly of both sexes. Among human subjects, 27.3% (95% CI, 20.9-33.2) showed evidence of prior leptospire infection. The seroprevalence observed here falls within the range of seroprevalences (8.0% - 42.6%) detected among a study of pregnant women in 10 English-speaking Caribbean nations.[20] The prevalence found in this community is slightly lower than the one-year observed incidence of 35.4%

observed in an urban slum in Brazil (95% CI, 30.7–40.6). Serogroup Icterohaemorrhagiae was the most common among humans and rodents in this study, and also has been common among animals on other Caribbean islands. [21] Seropositivity indicates exposure to bacteria within a time period of approximately the preceding 6 months to 1 year, but does not confer any information on occurrence of clinical infection. The evidence here confirms that pathogenic leptospires are circulating in this community, which suggests that leptospiral disease poses a serious health burden in this community and indicates that leptospiral disease occurrence in this area must be monitored for more closely.

The presence of the same strains of leptospires in humans, rodents, and the urine isolate suggests that leptospires are actively circulating in the community. Serogroups *L. interrogans* Icterohaemorrhagiae and *L. borgpetersenii* Ballum were identified in the rodents as well as in the humans. While these results are limited by the small number of rodents trapped, MAT testing of humans and rodents against the serogroup Ballum strain isolated from the rodent confirm the validity of the results and indicate that this pathogenic strain is circulating actively in the community.

Structural factors in the area, such as the Caño Martin Peña canal, could be important contributors to the spread of disease. Proximity of residence to the Caño Martin Peña canal was a significant predictor of leptospirosis infection both independently ($P = 0.0211$) and after accounting for household flooding and block grouping effects, which suggests that the canal could be a key source of transmission. Previous studies in CMP also showed that living near the canal was associated with increased diarrheal illness.[22] In order to mitigate the environmental hazards associated with the canal, the US Army

Corps of Engineers, in conjunction with the ENLACE community organization developed a plan in 2012 for the dredging of the canal to restore water flow and to protect the land rights and health of the residents of the surrounding neighborhood.[23] In 2014, a health impact assessment determined that the canal posed substantial health risks to nearby residents in terms of both chemical and biological exposures, and concluded that the US Army Corps of Engineers plan for dredging of the canal should be funded by the governments of Puerto Rico and the Federal Government and completed as soon as possible.[13] Despite these recommendations, the canal remains stagnant and the dredging project is not yet underway. The results from this study emphasize the role of the canal as a likely reservoir for transmission, and contribute to the body of evidence suggesting that this structural risk must be mitigated.

Other household attributes and individual risk factors were not found to be as clearly associated with disease as was proximity of the home to the canal. Previous studies had shown that dengue infections were clustered around flood areas and illegal dumpsites in this community, but in this study of leptospirosis, flooding was actually shown to be protective against infection ($P= 0.006$) and there was no association between proximity to a dump site and leptospirosis infection.[24] Reported street flooding was also shown to be protective against exposure in this study, but this trend was not significant ($P = 0.52$). Living in a house that flooded was still significantly protective against exposure in a mixed model controlling for block grouping, distance of the home from the canal, age, and sex (OR: 0.12, 95%CI: 0.04 – 0.37). However, a cross-sectional design sampling at only one point in time may not be appropriate for assessing seasonal risk factors like flooding, which can vary in severity between months or years. Since

flooding was measured through self reporting, it is possible that the association observed here represents a proxy of attitude toward flooding or that there is unmeasured confounding contributing to the observed effect. Sensitivity analyses including univariate and multivariate models adjusted for different grouping effects all detected similar associations. A study in Thailand also found an inconsistent relationship between incidence of leptospirosis and flooding across years and regions, further suggesting that in areas of recurrent flooding, flooding and leptospirosis may not be as interrelated as previously thought.[25]

No work-related exposures were identified as significant predictors of infection. Though suspected household exposures such as sighting of rats and presence of dogs chicken coops were common, there was no strong association with such animal-related exposures and infection.

This study had several limitations. Our study was limited by the cross-sectional design of the seroprevalence survey. Risk exposures may have changed from the time that the prior infection occurred to the time of survey, and migration could be occurring into and out of the study area. Now that leptospirosis has been confirmed as a serious problem in this community, a prospective study could be conducted to ascertain incidence of infection and disease and to obtain more reliable estimates on rates and risk associations. Many men and younger people could not be enrolled in the study because they were at work during data collection hours, even on the weekends. However, given that leptospirosis is commonly considered to affect young men the most, this suggests that our seroprevalence estimate from this study is quite possibly an underestimate of the true burden of disease in the community, strengthening the argument that further

investment and study are needed in this area. While this study only examined one community of San Juan, our findings suggest that other lower income areas of Puerto Rico that surround waterways could face similarly high risk of leptospirosis.

In conclusion, the results of this study show that there are high levels of exposure to leptospirosis in this population, supporting that leptospirosis is often misdiagnosed in this area. Rodents served as the main carrier of disease. These results should be used to inform public health policy, including increasing healthcare provider awareness about leptospirosis to improve diagnosis, as well as increasing efforts to clean and dredge the canal to reduce disease.

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Table 1: Distribution of Serogroups against which participants had the highest titers

Serogroup	Number of Subjects (N=55*) (% Positive)	Median (range) of reciprocal of positive titers
Mixed[a]	19 (34.5)	N/A
Icterohaemorrhagiae	12 (21.8)	100 (50 – 200)
Autumnalis	6 (10.9)	200 (50 – 400)
Ballum	4 (7.2)	100 (50 – 200)
Mini	4 (7.2)	100 (50 – 100)
Hebdomadis	3 (5.4)	100 (50 – 100)
Tarassovi	3 (5.4)	50 (50 – 100)
Bataviae	1 (1.8)	50 (50 – 100)
Canicola	1 (1.8)	50 (50 – 100)
Cynopteri	1 (1.8)	50 (50 – 100)
Manhao	1 (1.8)	100 (50 – 200)
Total Positives	55	N/A

*55 out of 202 subjects (27.2%) were seropositive

[a] In cases in which the sample had the same highest titer for several serogroups, the final serogroup result was defined as mixed

Table 2: Associations between study variables and seropositivity, adjusted for block groupings

Characteristic	N	Seropositive N (%) or Mean (95% CL Mean)	N	Seronegative N (%) or Mean (95% CL Mean)	P-value*
Individual risk factors					
Age (years)	54		143		0.1304
5-14		6 (11.1)		13 (9.1)	
15-29		8 (14.8)		17 (11.9)	
30-44		16 (29.6)		20 (14.0)	
45-59		9 (16.7)		40 (28.0)	
≥60		15 (27.8)		53 (37.1)	
Female Sex	54	26 (48.2)	148	54 (36.5)	0.2095
Highest Education	46		133		0.3494
Grade School or less		11 (20.0)		48 (32.2)	
High school		15 (27.3)		28 (18.8)	
College		20 (36.4)		57 (38.5)	
Household-related environment					
Distance from canal (m)	55	200.7 (171.5-230.0)	147	246.6 (225.7-267.5)	0.0211
Reported mud contact	44	11 (25.0)	134	24 (17.9)	0.4811
Reported Home Flooding	40	5 (12.5)	119	47(39.5)	0.006
Reported Street Flooding	50	22 (44.0)	139	78 (73.5)	0.3690
Household-associated behavioral exposures					
Walk barefoot at home	45	25 (55.6)	134	69 (51.5)	0.5917
Wear boots in floodwater	18	10 (55.6)	79	38 (81.4)	0.5707
Reservoir-related exposures					
Rats Sighted at home	45	39 (86.7)	133	111 (83.4)	0.5829
Rats Sighted at home in day	40	17 (42.5)	113	46 (40.7)	0.9703
Dog at home	55	49 (89.1)	148	132 (89.2)	0.8994
Chicken Coop	40	15 (37.5)	135	54 (40.0)	0.8575
Occupation-related exposures					
Employed	45	23 (51.1)	123	44 (73.2)	0.2245
Construction work	55	5 (9.1)	147	5 (3.4)	0.3045
Reported mud contact	36	21 (58.3)	93	53 (57.0)	0.7160

***** Student's t-test was used for continuous variables. A generalized linear mixed model with a Poisson distribution with random effects for block groupings was created to identify the individual effect of factors on risk of infection and to account for clustering within blocks.

Table 3. *Leptospira* load and identification in kidney specimens.

Rodent species	Animal	Leptospira concentration (Log_{10} GC/g)	secY identification	MLST type	MAT Highest Titer
Mus musculus	M1	8.93	99% *L. borgpetersenii*	N/A	Mixed
	M2	8.71	99% *L. borgpetersenii*	149	ND
	M3	8.50	99% *L. borgpetersenii*	149	Mixed
	M4	8.11	99% *L. borgpetersenii*	ND	Mixed
	M5	7.43	99% *L. borgpetersenii*	ND	Mixed
Rattus norvegicus	N1	5.60	99% *L. borgpetersenii*	149*	Icterohaemorrhagiae
	N2	3.02	Unsuccessful	ND	Icterohaemorrhagiae
	N3	negative	N/A	N/A	ND
Rattus rattus	R1	3.16	Unsuccessful	ND	ND
	R2	4.34	99% *L. borgpetersenii*	149*	Mixed
	R3	5.88	100% *L. interrogans*	Unsuccessful	Icterohaemorrhagiae
	R4	6.23	100% *L. interrogans*	17*	Icterohaemorrhagiae
	R5	negative	ND	ND	Negative
	R6	negative	ND	ND	Mixed
	R7	negative	ND	ND	Ballum
	R8	negative	ND	ND	Icterohaemorrhagiae
	R9	negative	ND	ND	ND
	R10	negative	ND	ND	ND

ND = not determined; *sequence type obtained from partial allelic profiles

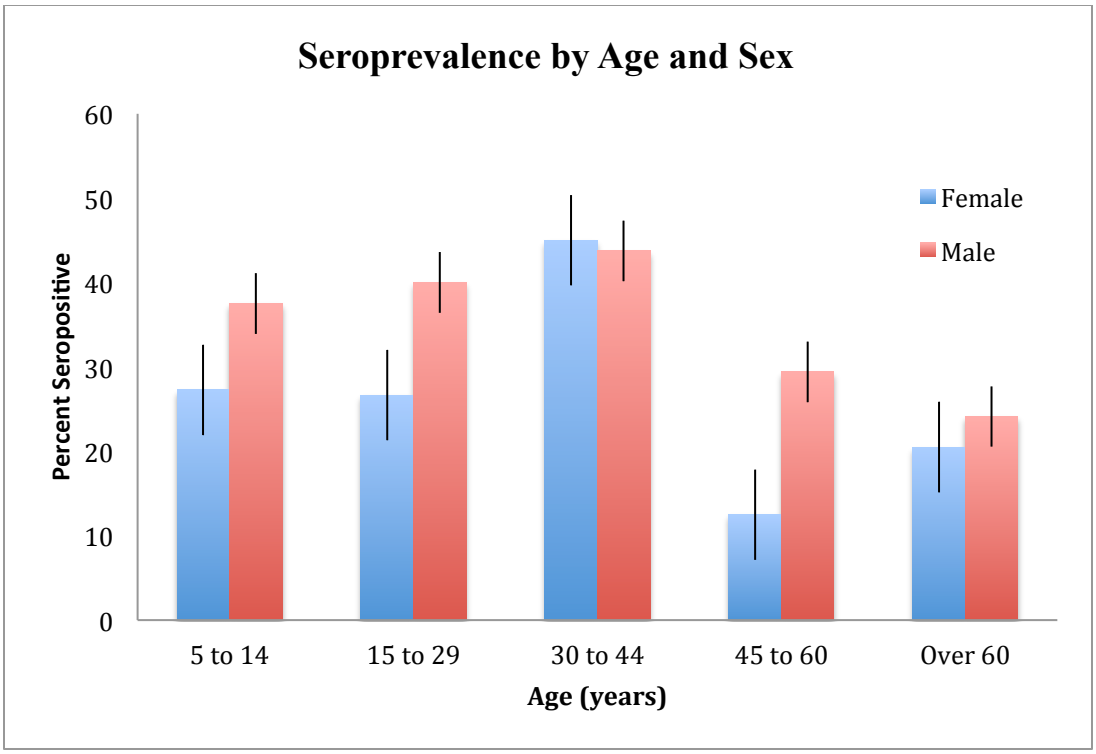
Table 4: Multivariable risk factors for leptospiral infection, adjusted for block groupings

Characteristic	OR (95% CI)*
Distance from canal (per 1m increase)	0.994 (0.990 – 0.998)
Reported Household Flooding	
Yes	0.12 (0.04 – 0.37)
No	ref
Age (per 1 year increase)	0.995 (0.976 – 1.013)
Female Sex	0.85 (0.37 – 1.95)

*A generalized linear mixed model was fit in Glimmix using random effects for block grouping and including home flooding and distance from canal as covariates, controlling for age and sex.

Figure 1: Seroprevalence of human subjects by age and sex, with 95% confidence intervals

Figure 2: Caño Martin Peña neighborhood in Puerto Rico a) Satellite image map of the four sampled census tracts showing the canal as a purple line, the location of seropositive subjects as orange circles, seronegative subjects as green triangles, and rodent trapping locations as red circles b) Location of Cano Martin Pena community as a dark blue rectangle within the city of San Juan, Puerto Rico c) A representative photograph demonstrating clogging of the canal within the community


Seroprevalence by Age and Sex
Percent Seropositive
60
50
40
30
20
10
0
Female
Male
5 to 14
15 to 29
30 to 44
45 to 60
Over 60
Age (years)


a
b
c

Appendix 1: Panel of Strains used in microscopic agglutination test for prior leptospire infection in human and rodent samples

Species	**Serogroup**	**Serovar**	**Strain**
L. interrogans	Australis	Bratislava	Jez Bratislava
L. interrogans	Autumnalis	Autumnalis	Akiyami A
L. borgpetersenii	Ballum	Castellonis	Castellon 3
L. borgpetersenii	Ballum	Ballum	Mus 127*
L. interrogans	Bataviae	Batavie	Van Tienen
L. interrogans	Canicola	Canicola	H. Ultrecht IV
L. weilii	Celledoni	Celledoni	Celledoni*
L. kirschneri	Cynopteri	Cynopteri	3522C
L. interrogans	Djasiman	Djasiman	Djasiman*
L. kirschneri	Grippotyphosa	Grippotyphosa	Duyster*
L. interrogans	Hebdomadis	Hebdomadis	Hebdomadis
L. interrogans	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
L. interrogans	Icterohaemorrhagiae	Copenhageni	M 20
L. interrogans	Icterohaemorrhagiae	Copenhageni	L1 130*
L. weilii	Javanica	Coxi	Cox
L. noguchii	Louisiana	Louisiana	LSU 1945
L. alexanderi	Manhao	Manhao 3	L 60
L. borgpetersenii	Mini	Mini	Sari
L. noguchii	Panama	Panama	CZ 214 K
L. interrogans	Pomona	Pomona	Pomona
L. interrogans	Pyrogenes	Pyrogenes	Salinem
L. alstoni	Ranarum	Pingchang	80-412
L. interrogans	Sejroe	Hardjo	Hardjoprajitno*
L. interrogans	Sejroe	Wolffi	3705
L. biflexa	Semaranga	Patoc	Patoc 1*
L. santarosai	Shermani	Shermani	1342 K
L. borgpetersenii	Tarassovi	Tarassovi	Perepelitsin
L. kmetyi	Tarassovi	Malaysia	Bejo-Iso9
L.borgpetersenii	Ballum	Not Determined	CMP Isolate

*Strains that showed reactivity to none of the first 100 random human sera samples tested were removed from the screening panel for humans. Rodent sera were tested against all 29 strains.