Effect of sewerage on the contamination of soil with pathogenic *Leptospira* in urban slums

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ABSTRACT

Leptospirosis is an environmentally-transmitted zoonotic disease caused by pathogenic *Leptospira spp.* that affects urban and rural poor communities world-wide. In urban slums, leptospirosis is associated with precarious sanitary infrastructure. Yet, the role of sewerage in the reduction of the environmental contamination with pathogenic *Leptospira* has not yet been explored. Here, we conducted a survey of the pathogen in soils surrounding open and closed sewer sections in six urban slums in Brazil. We found that the presence and concentration of pathogenic *Leptospira* was lower in soils adjacent to conventionally closed sewers, when compared to their open counterparts. However, no difference was observed in community closed sewers. We also found that human fecal markers (BacHum) were positively associated with pathogenic *Leptospira* even in closed sewers and that rat presence was not predictive of the presence of the pathogen in soils suggesting that site-specific rodent control may not be sufficient to reduce the environmental contamination with *Leptospira*. Overall, our results indicate that sewerage expansion to urban slums may help reduce the environmental contamination with the pathogen and therefore reduce the risk of human leptospirosis.

Keywords

Leptospirosis, sewer, public health, environment, fecal pollution

Synopsis

Sewerage construction in urban slums may reduce the presence and concentration of pathogenic *Leptospira*, thus decreasing the risk of human exposures.

INTRODUCTION

Leptospirosis is a neglected zoonotic disease that affects urban and rural communities worldwide[1] with an estimated annual burden of over a million cases and approximately 60,000 deaths.[2] Its clinical manifestations range from asymptomatic or a mild flu-like illness to severe disease such as Weil's disease and pulmonary hemorrhagic syndrome for which fatality rates are higher than 10% and 50%, respectively.[3,4] Leptospirosis is caused by pathogenic spirochetes from the genus *Leptospira*. Pathogenic *Leptospira* thrive in the kidneys of a wide variety of animals, some of which are chronical carriers, and are released with the urine into the environment at high concentrations[5,6] where they can survive for extended time.[7,8] Human infection occurs through contact with previously contaminated water and soil or by exposure of cuts and abraded skin with animal urine, making leptospirosis an environmentally-transmitted disease.[1]

Leptospirosis has historically been an occupational disease related to livestock raising, mining, rice farming and other agricultural activities,[9] but in the last 30 years it has emerged as an epidemic in urban communities surrounding cities in developing countries.[10–13] In these neglected settings, poverty, precarious housing and trash accumulation create the ecological conditions for the proliferation of rodents, particularly *Rattus norvegicus*, which are the primary reservoirs of pathogenic *Leptospira* in urban environments.[14,15] Extreme weather events and seasonal periods of heavy rainfall increase the presence of the pathogen in the environment[16,17] and the likelihood of human exposure to contaminated water, soil and mud due to inadequate sewer and storm drainage infrastructure.[18,19] Indeed, cross-sectional and prospective epidemiological studies have identified open sewers and drainage as risk factors for *Leptospira* infection in urban slums.[19–22] As the population living in urban slums is predicted to reach 2 billion by 2025[23], the burden of leptospirosis is only expected to increase.[18] There is, therefore, an urgent need to develop control measures for leptospirosis in resource-poor urban settings.

Sanitary interventions to close open sewers are an alternative to reduce exposures to environmental sources of *Leptospira* [16,24], given the lack of efficacious vaccines for human use [1,25] and the limited success of rodent control strategies due to regrowth after extermination [26,27]. Sewerage construction is widely recognized to reduce the incidence of viral, bacterial and parasitic diseases [28–30]. However, its effect on the reduction of pathogenic *Leptospira* contamination has not been examined. Here, we aimed to determine the effect of conventional and community-based sewer closings in the environmental contamination with pathogenic *Leptospira*. To this end, we performed a cross-sectional study in soils surrounding open and closed sewer sections in six Brazilian urban slums. The evaluation of the effect of sewerage in preventing environmental contamination with the pathogen is critical to inform public health interventions aimed to reduce the burden of leptospirosis in these neglected urban communities.

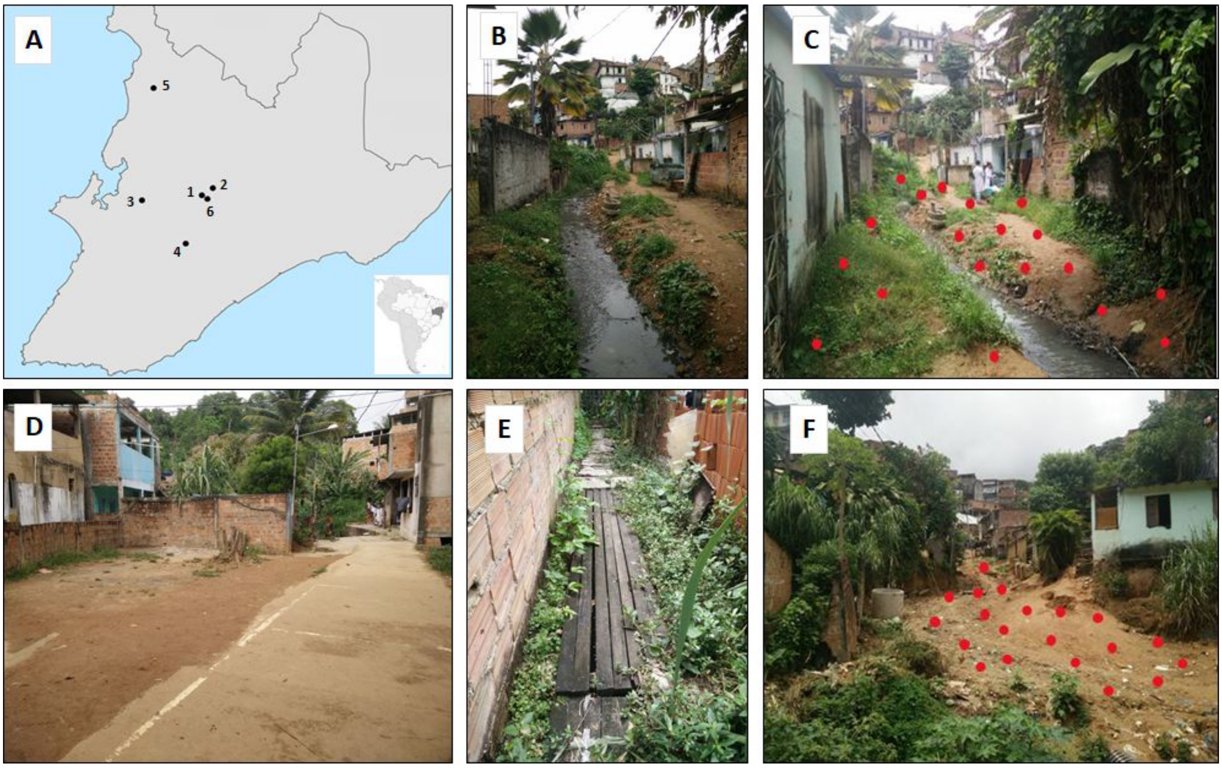
MATERIALS AND METHODS

Study sites

We conducted this study in six sites located in five urban slum settlements (*favelas*) in the periphery of the city of Salvador (Brazil). The incidence of severe leptospirosis in urban slums in Salvador is ca.19.8 cases per 100,000 inhabitants. [20] The communities studied were Pau da Lima (sites 1 and 6), Sete de Abril (site 2), Campinas de Pirajá (site 3), Tancredo Neves (site 4) and Nova Constituinte (site 5) (Fig. 1A). All these slums have similar characteristics of poverty, overcrowding, marginalization, poor quality housing and lack of reliable sanitation infrastructure than other slum settlements in Brazil and other developing countries. [23,31]

Specifically, the precarious sanitation system results in untreated sewage and storm water drainage flowing through open sewers across these communities. In each community, we selected one site containing a sewer with contiguous open and closed sections (Fig 1B and 1D). Closed sections were classified as conventional or community-based depending on the type of closing. Conventional closings (sites 1, 2, 3 and 4) were built by the local government sewage company by digging trenches and placing sewer mains to which every house drain was connected (Fig 1D). Conventional closings isolated the sewer and prevented sewage from contaminating the surrounding environment. Community closings (sites 5 and 6) had been performed informally by the local dwellers and consisted of wood planks or concrete boards placed on top of the open sewer (Fig 1E). Community interventions prevented major spills from the sewer but did not avoid leaking or major overflowing during rainfall events.

Figure 1. Distribution of sampling sites in the study area and typology of sewer closing. **A)** Map of the city of Salvador (Brazil) with the locations of the six urban slum communities where soil collections were performed: Pau da Lima (sites 1 and 6), Sete de Abril (site 2), Campinas de Pirajá (site 3), Tancredo Neves (site 4) and Nova Constituinte (site 5). **B)** An open sewer section in site 1. **C)** Soil sampling points (red dots) in soil at open sewer section. **D)** Conventionally closed sewer section in site 2. **E)** Community closed sewer section in site 5. **F)** Soil sampling points (red dots) in soil at closed sewer section



Sampling design and sample collection

At each site, open and closed section areas containing exposed soil within a 12 m distance to the main sewer were demarcated, georeferenced and entered in a GIS database. Polygons of 150 m^2 to 220 m^2 were drawn in each closed and open area and 24 collection points were randomly selected using a packing density of 0.4 with corresponding minimum distances between collection points for each area. (Fig 1C and 1F). Because of size constraints, only 16 collection points were selected in site 6. In total 272 collection points were selected, 136 in open and 136 in closed sewer areas.

Samples were collected in the first week of December of 2018. At each collection point, an area of ~400 cm^2 was cleared from surface rocks and vegetation debris, and ~25g of subsurface soil were collected, stored in aseptic containers, transported to the laboratory and processed within 4h of collection as described previously with minor modifications.[32] Briefly, 40 mL of sterile double-distilled was added to each 5g sample and shacked with a horizontal vortex adaptor at maximum speed for 2 min. Samples were centrifuged at 100 rcf for 5 min, the supernatant recovered and centrifuged at 12,000 rcf for 20 min at room temperature. The supernatants were discarded, and the pellets frozen at -20˚C. In addition to the soil samples, two paired 40-mL sewage samples were collected at the end of the closed and open sections in each site. Sewage samples were processed as described previously.[16]

Quantification of pathogenic *Leptospira* and human fecal markers.

DNA was extracted from the frozen pellets within 3 days after processing using DNA Easy PowerSoil kit (Qiagen) in batches of 20 samples and stored at -80 °C. An extraction blank (sterile double-distilled water) was included to each batch to control for cross-contamination.

Pathogenic *Leptospira* was quantified using a TaqMan assay targeting the *lipL32* gene as described previously.[32] To determine the levels of human fecal contamination, we used the BacHum TaqMan qPCR assay.[33] Calibration curves were included in each qPCR plate for with concentrations of standard ranging from 2×10^2 to 2×10^9 GEq/mL. Samples were run in duplicate and included non-template controls in each plate row to control for contamination. qPCR inhibition was monitored using a an Internal Amplification Control (IAC) plasmid in singleplex reactions as described previously [16] for *lipL32* and testing at least two sample dilutions for BacHum. For more details on cycling parameters, primer, probe and bovine serum albumin (BSA) concentrations, calibration curves and tests for inhibition, see the Supporting Information.

Rat activity monitoring

To evaluate the rat presence in the sampling sites during soil collections, we used a track plate method that had previously showed high correlation with rat infestation measures and trapping of rats to population exhaustion approaches.[34] Forty-eight track plates were placed in each of the demarcated areas described above on the day of soil collection. Plates were randomly distributed within each polygon with a packing density of 0.4 with corresponding minimum distances between them (1.33 ± 0.73 m). Each site contained 96 track plates (48 in the surroundings of the open section of the sewer and 48 in the closed section), for a total of 576 plates. Track plates were evaluated daily over the course of two days for evidence of rat activity through the identification of footprints, scrapes, and tail slides and scored using a binary variable (presence/absence of rat marks on a plate) and a continuous variable (the intensity of marks on plates).[34] In addition, environmental rodent surveys were carried out at each sampling site by looking for variables associated with rodent infestation and water or harborage sources for rodents: pavement, soil, mud vegetation, trash, food , water, building material, rubble, others animals and rat feces.[35]

Data treatment

Samples were considered positive when both qPCR replicates showed amplification up to a C_T of 40. Samples with a single positive reaction were submitted to an additional qPCR run in duplicate. If in this second run the sample amplified in either of the replicates, it was considered positive. The genomic equivalents (GEq) per reaction in all positive qPCR replicates were averaged, normalized by the amount of soil or water processed, and $\log_{10}$-transformed to obtain concentrations in $\log_{10}$ GEq/g or mL. For the purpose of statistical analysis, soil samples with concentrations below the limit of detection were considered to have a concentration equivalent to the limit of detection of the lipl32 qPCR assay in soil samples (2GC/g). [32]

Statistical analysis

We built mixed generalized linear models (GLMMs) with binomial and gamma error structure to investigate the probability of presence (binomial) and concentration of pathogenic *Leptospira* in soil (continuous in $\log_{10}$) and their association with sewer status (open /closed) and type of closing (open, conventional or community-based). [36] We also included other covariates such as distance to the sewer, soil moisture, presence of rats and human fecal markers (BacHum), and a randomization factor for the sampling site. The modeling approach was carried out in two stages. First, we built univariate models between all the variables and added an interaction structure between them to understand how the presence and concentration of *Leptospira* in soil varied. Variables with a p-value below 0.1 in univariate analyzes were included in the multivariate analyzes, subsequently performed. Various multivariate statistical models were generated, and the model with the lowest AIC (Akaike's Information Criterion) and ΔAIC <2 was selected as the best model using the *dredge ()* function of the R MuMIn package. [37,38] We estimated the odds ratios (ORs) associated with the probability of *Leptospira* presence in soil and the rates (β coefficients) for the *Leptospira* concentration model in soil. The analyzes were performed in R 3.3.1 [39], and we applied a significance level of $p < 0.05$.

RESULTS

Presence of *Leptospira* DNA in soil samples

We collected a total of 272 soil samples and 24 sewage samples in the six sites studied and tested them for the presence of pathogenic *Leptospira* DNA. Overall, 68 soil samples (25.0%) were positive for *Leptospira* DNA with more samples positive in soils surrounding the open sewer sections (31.6% [24.4 39.9%, 95% CI] than in their closed counterparts (18.4% [12.7% 26.8%, 95% CI] (Table 1). Among the 68 positive samples, the geometric mean concentrations and count range of *Leptospira* DNA was 3.3 [2.00-1.62×10^3] GEq/g and 4.2 [2.0 – 52.6] GEq/g in open and closed sections, respectively (Table 1, Fig. 1 and Suppl. Fig 1). The highest proportion of positive samples was detected in the open section of Site 1 (54.2%, 13 of 24), whereas the lowest was found in the closed section of Site 2 (0%, 0 of 24) (Table 1). Interestingly, while we observed a relative reduction in the percentage of positive samples in most conventionally closed sites (sites 1, 2 and 3) compared to open sites, no reduction was observed in community closed sites (sites 5 and 6).

Table 1. Occurrence and concentration of pathogenic *Leptospira* in soils surrounding open and closed sewers in the six Brazilian urban slums. Closed sewers are classified based on the type of closing: conventional or community-based.

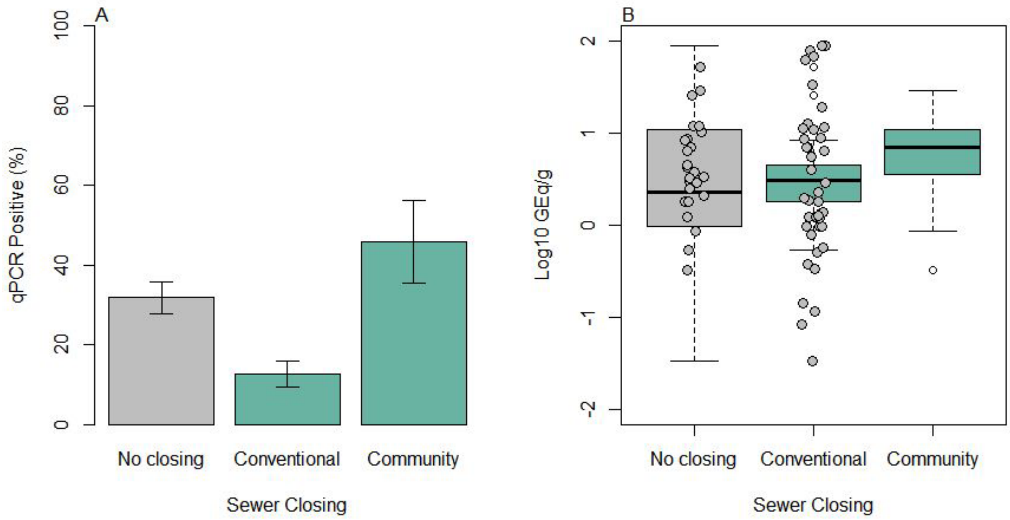
Site and type of closing	**Pathogenic *Leptospira* positivity rate (n and %)[a]**		**Pathogenic *Leptospira* concentration (mean $\log_{10}$ and SD)**	
	Open sewer	**Closed sewer**	**Open sewer**	**Closed sewer**
Conventional				
Site 1 – Pau da Lima 1	13 (54.2%)	2 (8.3%)	1.04 (0.70)	0.57 (1.19)
Site 2 – Sete de Abril	3 (12.5%)	0 (0.0%)	0.61 (0.82)	0.00 (0.00)
Site 3 – Campinas de Pirajá	8 (33.3%)	3 (12.5%)	-0.08 (0.74)	0.54 (0.04)

Site 4 – Tancredo Neves	4 (16.7%)	5 (21.7%)	1.04 (1.99)	0.48 (0.30)
Total conventional	28 (29.1%)	10 (10.5%)	0.67 (1.05)	0.51 (0.44)
Community				
Site 5 – Nova Constituinte	11 (45.8%)	11 (45.8%)	0.20 (0.62)	0.70 (0.56)
Site 6* – Pau da Lima 2	4 (26.7%)	4 (25.0%)	0.35 (0.45)	0.67 (0.72)
Total community	15 (38.4%)	15 (37.5%)	0.24 (0.57)	0.69 (0.57)
Overall	43 (31.9%)	25 (18.5%)	0.52 (0.93)	0.62 (0.52)

[a]24 samples were collected in open and closed areas from sites 1-5.

*16 samples were collected in open and closed areas from site 6.

Fig 2. Distribution of pathogenic *Leptospira* based on lipl32 qPCR in soils surrounding open and closed sewers. **A)** Occurrence by the type of sewer closing (mean percentage and standard error). **B)** Overall concentration of pathogenic *Leptospira* by the type of sewer closing (geometric mean and 95% confidence interval). Open sewers are denoted in gray and closed sewers in green.



Presence and concentration of human fecal pollution markers

We detected the human fecal pollution markers (BacHum) in 56.3% (153 of 272) of the soil samples collected (Suppl. Table 1). The presence of the marker was slightly higher in open than in closed areas of the sewers (58.8% [50.4%-66.8%] and 53.3% [45.0%-61.4%], respectively). The highest proportion of positive samples occurred in the open sections of sites 1 and 6 (91.7% and 100%, respectively), whereas the lowest was found in the open and closed section of site 4 (8.3% and 16.7%, respectively). Among the 153 positive samples, the geometric mean concentrations and count range of BacHum was 3.04×10^3 [21.4-2.41×10^7] GEq/g and 1.26×10^3 [66.4 – 8.06×10^3] GEq/g in open and closed sections, respectively (Supplemental Table 2).

Presence of rats

We observed a higher presence and activity of rats as measured by tracking boards in the closed sections (12.2% and 29.2%; $p=0.0139$) when compared to the open sections of the sewers (9.3% and 18.1%; $p<0.001$). Tracking plates placed within the area of the closed section of the sewer had higher rat presence, 12.2 % (±8.3) vs. 93% (±9.8) $p=0.0139$, and higher percent rat activity, 29.2% (±6.9) vs. 18.1% (±19.2) p<0.001, than the tracking plates placed near the open section of the sewer. We did not find significant differences between the open/closed status of the sewer and the number of animals (p=0.4484), number of rat holes (p=1.0000), pavement (p=0.4902), soil (p=0.1138), mud (p=0.5271), vegetation (p=1.0000), trash (p=1.0000), food (p=0.5271), water (p=1.000), building material (p=0.4902), rubble (p=0.5271), and rat feces (p=1.000).

Sewage samples

Pathogenic *Leptospira* was present in 17 of 24 the sewage samples collected with a geometric mean and count range of 124 [20-1,545] GEq/mL. In all collection sites (before and after sewage closing), at least one of the two paired samples collected was positive, indicating that sewage was a frequent source of pathogenic *Leptospira* in all sites.

Predictors of *Leptospira* DNA presence and concentration

The univariate models found significant associations between the presence and concentration of pathogenic *Leptospira* in soil with the status of the sewer (open/closed), the type of closing, distance to other nearby open sewers, rat activity and the concentration fecal human markers (BacHum) (Supp. Table 2 and 3). However, only two covariates remained significant in the multivariate final models: type of sewer closing and presence of BacHum fecal pollution markers (Table 2 and Supp.Table 4). First, soil samples collected in areas surrounding sewers closed by the local government were more than 3 times less likely (inverse OR 3.44, 95% CI: 1.66-8.33) to contain pathogenic *Leptospira* than soils collected in open areas overall. In contrast, the presence of pathogenic *Leptospira* was not significantly different in soils surrounding community closed sewers than in those adjacent to open sewers. Similarly, the logistic model using *Leptospira* concentration as outcome indicated that soils surrounding conventionally closed sewers contained a lower load of pathogenic *Leptospira* (0.82 $\log_{10}$ units less, or approximately 6 times less). Furthermore, the logistic model showed that BacHum markers were significantly associated with the presence of pathogenic *Leptospira*. For every $\log_{10}$ unit increase in BacHum concentration, the chances of finding a positive *Leptospira* sample increased by 15%. Likewise, the concentration of pathogenic *Leptospira* in positive samples was higher in those samples that also contained BacHum markers (Table 2). Notably, none of the other variables included in the model (rat presence and activity, soil moisture, distance to open or closed sewer and proximity to other open sewers) were found to be significantly associated with pathogenic *Leptospira* in the multivariate models. In summary, our model revealed that type of closing and BacHum markers were important predictors of presence and concentration of pathogenic *Leptospira* in soil.

Table 2- Final multivariate logistic and linear mixed models on the probability of finding a positive sample and $\log_{10}$ concentration for *Leptospira* DNA. (**) p = 0.001 (***) p = 0.0001

	Logistic model for probability		**Model for concentration**	
Predictors	OR	CI	Estimates (β)	CI
(Intercept)	0.40***	0.21 – 0.70	-2.6***	-3.07 – -2.16
Type of closing				
No closing (Ref.)	_	_	_	_
Conventional	**0.29*** **	**0.12 – 0.60**	**-0.82** **	**-1.33 – -0.30**
Community	1.09	0.46 – 2.55	0.19	-0.54 – 0.93

Fecal human markers				
Concentration fecal human markers (log_{10} GE/mL)	**1.15****	**1.04– 1.26**	**0.11****	**0.04 – 0.18**

DISCUSSION

In this study, we compared the presence and concentration of pathogenic *Leptospira* in soils surrounding open and closed sewer sections in six Brazil urban slums. We found that pathogenic *Leptospira* occurred in both areas but was more prevalent in soils adjacent to open sewer sections, although the concentration was generally low. More importantly, our results show that soils in conventionally closed sewers have a reduced presence of the pathogen, as opposed to community-closed sewers. These results have important implications for future public health and sewerage development in urban slums.

The soil contamination with pathogenic *Leptospira* was lower in soils adjacent to conventionally-closed sewers than open sections, but no reduction was observed in community-based closings (Table 1 and Fig. 2). Conventional sewer closings completely canalize sewage, isolating it from the surrounding environment and preventing spills and overflow during heavy rainfall events. Since sewage is a recognized source of *Leptospira* as evidenced by this and previous studies [16,24,40], its canalization may eliminate spillage contaminations in soil. However, the imperfect closure of community-based interventions could still allow sewage to contaminate adjacent soils. Moreover, despite the reduction observed in conventionally closed sections, the pathogen could still be detected in 3 of the 4 sites sampled. This suggests that the presence of pathogenic *Leptospira* contamination in these soils may not have its origin exclusively in the adjacent open sewers.

We identified human fecal markers (BacHum) as a predictor of presence and concentration of pathogenic *Leptospira* in soils. A previous study in streams from Hawaii, also found a positive correlation of pathogenic *Leptospira* concentrations and fecal pollution markers (*Bacteroidales* and *Clostridium perfringens*) [41]. Considering that the major sources of human fecal pollution in this environment are open sewers, human fecal markers are likely a surrogate for the distance to the sewer in open sections. However, in closed sections, the correlation of BacHum and pathogenic *Leptospira* indicates that there are other sources of fecal pollution and of pathogenic *Leptospira*. Previous studies have hypothesized that intense rainfall events may mobilize pathogenic *Leptospira* and human pollution markers occurring in soils in higher elevated areas and transport them with the storm run-off to lower areas [32,42,43], where sewers are located. Notably, the construction of conventional sewers does not canalize storm water, and thus, run-off may still contribute to the contamination observed in conventionally closed sewer sections. Therefore, the association of pathogenic *Leptospira* and human fecal markers is likely a combination of the effect of sewer proximity and storm run-off. This may explain repeated contamination events in the areas surrounding the sewer, favoring *Leptospira* survival and increasing the risk of exposure of humans and animals to contaminated environments. [43,44]

The concentrations of pathogenic *Leptospira* in the collected soils were generally low in the six urban areas studied (mean $\log_{10} 0.56 \pm 0.8$ and $2.00\text{-}1.62 \times 10^3$ GEq/g) (Fig 1). This finding is consistent with previous studies that have reported low concentrations of the pathogen in soils and waters in high-risk environments. [16,24,32,45]. Besides the sewer and run-off contribution, the presence and concentration of the pathogen in soil is related to its survival and long-term persistence ability [8,46] which is affected by the soil type, composition and physicochemical characteristics. For instance, soils rich in nutrients such as iron, manganese, copper and nitrate have been shown to be a positive risk factor for the presence of *Leptospira*, just as wetter soils and basic pH can increase the survival of this pathogen [47–49]. Interestingly, a soil sample in site 4 contained a particularly high concentration of pathogenic *Leptospira* (1.62×10^3 GEq/g), which indicates that hot-spots of the pathogen occur in the urban slum environment. Yet, the highly heterogenic distribution of the pathogen in soil [32] and the cross-sectional nature of our sampling strategy may have prevented the identification of these high-concentration areas and determine their origin and temporal dynamics. Since the human infectious dose is still unknown, more studies are needed to determine the significance of these heterogenic distribution of the pathogen in human infection dynamics.

Unexpectedly, rat presence and activity were not important factors to predict the presence or concentration of the pathogen in soils. Rats are the main animal reservoir of pathogenic *Leptospira* in urban slums [5,50,51] and rat presence is commonly reported as a factor for leptospirosis infection. [35,52] Open sewers offer an ideal ecosystem for the proliferation of rodents by providing burrowing areas, access to water and food sources. Counterintuitively, our results suggest that the contamination of soils close to sewers is more related to the type of sewer closing than to the presence and activity of rats. Therefore, rat control strategies alone such as rodenticide campaigns, may not be effective in reducing the presence of the pathogen in the sewer environment [53,54] and should be combined with sewerage construction.

This study was limited by its cross-sectional design. Because *Leptospira* soil contamination may be variable over time and, specifically, around rainfall events, future prospective studies are needed to investigate the effect of sewer closing in the presence and concentration of the pathogen. In addition, the high heterogeneity of urban slum environments and diversity of community-based closings limit our ability to make wide generalizations of the effects observed in this study. Furthermore, although a higher environmental presence of pathogenic *Leptospira* is intuitively linked to a higher risk of infection, epidemiological studies are needed to determine how sewerage interventions and the reduction of the environmental burden of the pathogen affect the dynamics of leptospirosis infection and disease. These future studies will also need to determine whether community interventions, despite not reducing the environmental burden of *Leptospira*, may still decrease human infection. As community interventions are cheaper and easier to implement in neglected communities, more research is needed to understand their potential role in disease transmission.

Despite these limitations, taken together our results suggest that conventional sewer systems may be an important, but not exclusive strategy to reduce the presence and concentration of the pathogen in the environment. The closure of sewers could reduce the niches for the environmental distribution and dissemination of pathogenic *Leptospira* subsequently decreasing pathogenic *Leptospira* exposures in these neglected communities and eventually reducing human leptospirosis. This adds to the body of evidence that sewerage reduces exposures to a wide number of human pathogens and therefore, supports the expansion of sewer systems in urban slums to help decrease the burden of leptospirosis and other environmentally-transmitted infectious diseases.

Conflict of interests

The authors declare no competing financial interest.

Acknowledgements

We would like to thank the resident associations, community leaders and residents from Pau da Lima, Tancredo Neves, Sete de Abril, Nova Constituinte and Campinas de Pirajá.

Funding

This work was supported by the National Institutes of Health (grants F31 AI114245, U01 AI088752, R01 TW009504) and by the Wellcome Trust [218987/Z/19/Z]. Melany Curry was supported by a Downs International Health Student Travel Fellowship from Yale School of Public Health.

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