UNIVERSIDADE FEDERAL DA BAHIA – UFBA

INSTITUTO DE BIOLOGIA

Programa de Pós-Graduação em Ecologia: Teoria, Aplicação e Valores

Doutorado em Ecologia

FÁBIO NEVES SOUZA

CONECTANDO ROEDORES, AMBIENTE E HUMANOS NA DINÂMICA DE TRANSMISSÃO DA LEPTOSPIROSE URBANA: efeito de intervenções ambientais e sanitárias

Connecting rodents, environment, and humans of urban leptospirosis transmission dynamics: Effect of environmental and sanitation interventions

Salvador- Bahia-Brasil

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Tese apresentada ao Programa de Pós-Graduação em Ecologia: Ecologia: Teoria, Aplicação e Valores, como parte dos requisitos exigidos para obtenção do título de Doutor em Ecologia.

Orientador: Dr. Federico Costa

Coorientador: Dr. Cleber Cremonese

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Ficha catalográfica elaborada pelo Sistema Universitário de Bibliotecas (SIBI/UFBA),
com os dados fornecidos pelo(a) autor(a).

Neves Souza, Fábio
CONECTANDO ROEDORES, AMBIENTE E HUMANOS NA DINÂMICA DE TRANSMISSÃO DA LEPTOSPIROSE URBANA: efeito de intervenções ambientais e sanitárias / Fábio Neves Souza. -- Salvador-Bahia, 2023.
153 f. : il

Orientador: Federico Costa.
Coorientador: Cleber Cremonese.
Tese (Doutorado - Ecologia) -- Universidade Federal da Bahia, Programa de Pós-Graduação em Ecologia: Teoria, Aplicação e Valores-Instituto de Biologia (UFBA), 2023.

1. Contaminação ambiental. 2. Esgotamento sanitário. 3. Ecologia de doenças. 4. Leptospirose. 5. Intervenção. I. Costa, Federico. II. Cremonese, Cleber. III. Título.

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA: TEORIA, APLICAÇÂO E VALORES
UNIVERSIDADE FEDERAL DA BAHIA – INSTITUTO DE BIOLOGIA
Rua Barão de Geremoabo, s/n. Ondina – Salvador – Bahia – CEP. 40.170-000

COMISSÃO EXAMINADORA

Título de Tese: **Conectando roedores, ambiente e humanos na dinâmica de transmissão da Leptospirose urbana: efeito de intervenções ambientais e sanitárias**

Doutorando: **Fábio Neves Souza**

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HOMOLOGADO EM 25/04/2023

ATA DA SESSÃO PÚBLICA DO COLEGIADO DO PPG EM ECOLOGIA: TEORIA APLICAÇÃO E VALORES - INSTITUTO DE BIOLOGIA – UFBA

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Aos vinte e cinco dias do mês de abril de dois mil e vinte e três, a partir das 08h05min, por videoconferência, de acordo com o regimento geral da UFBA e com o regimento interno deste programa de pós-graduação, realizou-se a sessão pública de Defesa de Tese intitulada "**Conectando roedores, ambiente e humanos na dinâmica de transmissão da Leptospirose urbana: efeito de intervenções ambientais e sanitárias**". Foram iniciados os trabalhos da Comissão Examinadora, composta pelo professor Dr. Federico Costa (Presidente/orientador), Dr. Cleber Cremonese (coorientador), Dra. Claudia Muñoz-Zanzi, Dra. Christine Elizabeth Stauber, Dr. Carlos Roberto Franke, Dra. Patrícia Campos Borja e Dr. Hussein Khalil. O doutorando fez a apresentação oral de sua tese durante 50 minutos. Após o encerramento das arguições, às 10:25 horas, a Comissão Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese e em seguida pronunciou-se pela sua **Aprovação**, conforme parecer em anexo. Proclamados os resultados pelo Professor Doutor Federico Costa, Presidente da Comissão Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Comissão Examinadora, ao(s) vinte e cinco dias do mês de abril de dois mil e vinte e três (25/04/2023). Esta Ata será assinada pelos membros da Comissão Examinadora e deste Colegiado de curso, para compor o processo de emissão do diploma.

Salvador, 25 de Abril de 2023

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PARECER DA BANCA EXAMINADORA

Título de Tese: **Conectando roedores, ambiente e humanos na dinâmica de transmissão da Leptospirose urbana: efeito de intervenções ambientais e sanitárias**

Doutorando: **Fábio Neves Souza**

Orientador: **Prof. Dr. Federico Costa**

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A Comissão Examinadora, com base nos pareceres individuais em anexo, considera o trabalho de conclusão de curso:

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Dedico esta tese ao meu querido avô, que sempre me inspirou com sua sabedoria, perseverança e amor incondicional.

Agradecimentos

Agradeço à Universidade Federal da Bahia (UFBA) que me proporcionou o ambiente de estudo e crescimento profissional e acadêmico adequados, a Fundação de Amparo à pesquisa do estado da Bahia (FAPESB) pelo financiamento dos meus estudos.

Sou grato aos meus orientadores, Dr. Federico Costa e Dr Cleber Cremonese, que dedicaram seu tempo e esforços para me guiar neste trabalho de pesquisa e me ensinaram as habilidades e competências necessárias para ser um bom pesquisador. Serei eternamente grato a eles. Muito obrigado por acreditarem em mim.

Agradeço a todos os colegas e amigos do Instituto Gonçalo Moniz e do Instituto de Saúde Coletiva pelo apoio na execução e desenvolvimento desta tese. Em especial, quero agradecer a Fabiana Palma, Max Eyre, Michael Adedayo, Juliet Cerqueira, Jeorgia Rosado, Monique Silva e as minhas queridas alunas Roberta Coutinho, Maisa Aguiar, Ariane Goncalves e Camile Andrade. Chegar até este momento não teria sido possível sem a ajuda e o apoio de todos vocês.

Agradeço também à minha família pelo apoio incondicional, encorajamento e paciência durante os momentos difíceis. Seu suporte foi fundamental para eu me manter motivado e perseverante em minha jornada. Expresso aqui a minha gratidão e amor à minha companheira e amiga Mai, que foi essencial para manter meu equilíbrio emocional e psicológico (risos) em meio aos desafios.

Agradeço a todos os meus amigos, Ana Caroline, David Santana, Edilene Pestana, Juliana Macedo, Mailson Martins e Mellamy Marten, que me apoiaram com palavras de encorajamento, discussões tantos bobas quanto intelectuais, além de momentos de descontração que me ajudaram a manter um equilíbrio emocional.

Por fim, sou grato às comunidades e pessoas envolvidas neste estudo. Sem o envolvimento, apoio e receptividade de todos os envolvidos, este trabalho não teria sido possível. Agradeço a todos.

TEXTO DE DIVULGAÇÃO

Fábio Neves Souza

Em diferentes comunidades carentes nos centros urbanos do Brasil é comum ouvirmos: "calça o sapato para não pegar a doença do rato!!'', principalmente em áreas com inúmeros eventos de inundação e alagamentos devido às cheias dos esgotos abertos durante episódios de chuvas. Mas, qual o papel do esgoto na transmissão da doença do rato? Como o ambiente influencia no risco a esta doença?

A tal doença do rato é conhecida como leptospirose. Esta doença é causada pela bactéria leptospira que vivem dentro da maioria dos ratos de esgoto. A pessoa pode se contaminar pelo contato direto com a urina do rato ou pelo contato com água, lama ou terra contaminada por esta bactéria. É uma doença muito grave que acomete, principalmente, moradores de comunidades sem saneamento básico, pois nestas áreas a presença de lixo e esgoto a céu aberto torna o ambiente propício para a população de ratos e o espalhamento da bactéria na terra. Isso faz, com que as pessoas vivam mais próximos ao esgoto e, consequentemente, estejam em maior risco de ter leptospirose.

O controle desta doença ainda não é eficaz. Faz-se necessário intervenções públicas, governamentais ou comunitárias imediatas. Estas intervenções devem ser direcionadas para melhorias no saneamento, principalmente nas áreas com elevada concentração da pobreza e esgotos abertos, a fim de garantir a proteção da saúde e enfrentamento de doenças, como, por exemplo, a pandemia de Covid-19 que agravou e ampliou a pobreza e ocasionou mudanças ambientais com efeitos diretos na quantidade de ratos nestas comunidades. Intervenções em saneamento são caras, com necessidade de investimentos públicos contínuos para implementação e manutenção. Uma vez implementadas, devem ser avaliadas quanto aos reais impactos na melhoria ambiental e das condições de saúde humana. O fechamento dos sistemas de esgoto, que geralmente passam por áreas de baixa altitude, pode ser uma medida chave na redução de doenças ligadas ao saneamento, já que este tipo de intervenção somado a outras ações de melhorias do ambiente nas comunidades pode proteger os residentes da exposição a fontes de contaminação e reduzir a introdução de contaminação adicional da água de esgoto a montante dos corpos d'água. Este modelo de intervenção pode contribuir na diminuição no número de casos de leptospirose e outras doenças que tenham o esgoto inadequado como rota alternativa de transmissão, a exemplo das diarreias.

Apesar de hipotetizarmos que o fechamento do esgoto poderá reduzir o risco de contaminação por *Leptospira* e possível adoecimento por leptospirose, não temos um conjunto de dados científicos que avaliaram os impactos destas intervenções na redução dos elementos da transmissão da doença, quer dizer no espalhamento da bactéria no ambiente, na atividade dos ratos e na diminuição no número de casos da doença. Portanto, neste trabalho procuro compreender como diferentes intervenções sanitárias (canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos) poderia atuar na diminuição da transmissão da doença do rato. Assim, com este conhecimento, propor um diagnóstico da efetividade destas ações de saneamento em locais de precariedade social e elevado risco associado a problemas de saúde humana-ambiental. Nossos resultados revelaram que intervenções de fechamento de esgotos contribuem na redução das atividades dos roedores. No entanto, ações de fechamentos dos corpos d`água sem intervenções em saneamento, ou seja, serviços básicos urbanos de limpeza e drenagem mostrou-se sem efeito na redução na transmissão de *Leptospira* nos residentes. Apesar destes resultados, evidenciamos que os fechamentos dos esgotos contribuem para diminuição da carga da bactéria *Leptospira* no ambiente e estes achados permite propor novas questões direcionadas a compreender como diferentes intervenções de longa duração em saneamento afetam os mecanismos de transmissão da leptospirose. Em resumo, podemos concluir que comunidades empobrecidas, economicamente e com baixo acesso a serviços básicos urbanos, que recebam, pontualmente, intervenções baseadas unicamente em esgotamento não seriam suficientes para redução da transmissão do patógeno, mas talvez para a redução da doença. Portanto, são necessárias intervenções públicas de saneamento combinadas, com ampliação dos serviços básicos urbanos e com a participação e envolvimento dos residentes no conjunto das intervenções, para reduzir a carga de doenças e garantir uma justiça social e ambiental aos moradores destas comunidades historicamente marginalizadas.

SOUZA, F.N. Conectando roedores, ambiente e humanos na dinâmica de transmissão da leptospirose urbana: efeito de intervenções ambientais e sanitárias. 153 pp. Tese de doutorado em Ecologia – Instituto de Biologia, Universidade Federal da Bahia, Salvador, 2023

Resumo

A leptospirose continua a ser um grave problema de saúde nas comunidades urbanas empobrecidas dos países em desenvolvimento. A presença de corpos d`água poluídos e esgotos a céu aberto contribui para contaminação ambiental pela bactéria *Leptospira* e na abundância de roedores, representando a principal fonte de exposição dos residentes destas localidades. A não efetividade dos programas de controle de roedores e a ausência de intervenções sanitárias contribui para elevada incidência da infecção por *Leptospira*. Uma vez implementadas intervenções sanitárias e/ou de esgotamentos seus impactos deverão ser avaliados para compreender seus efeitos na transmissão de doenças, como no caso a leptospirose, e na saúde ambiental. Este trabalho, trata-se de um estudo para avaliar o impacto de intervenções sanitárias governamentais e comunitárias na carga ambiental da *Leptospira* no ambiente, na presença de ratos e na infecção por *Leptospira* na cidade de Salvador, Brasil. No **primeiro capítulo**, evidenciamos que intervenção em esgotamento reduz locais de refúgio e movimentação dos ratos diminuindo a chance da sua presença nestes locais. No entanto, a intervenção realizada não reduziu as taxas de incidência de infecção por *Leptospira*, uma vez que, a exposição dos residentes não variou entres os períodos antes e após a intervenção, limitando a compreensão do efeito da obra na carga da doença. No **capítulo dois**, mostramos que intervenções governamentais de fechamentos dos esgotos reduzem a carga de leptospira no ambiente. A canalização e tamponamentos dos esgotos e corpos d'água evitam transbordamento durante as fortes chuvas fazendo com que toda a água seja direcionada, reduzindo a contaminação do solo. Intervenções sanitárias integradas, com a comunidade local em conjunto a outras ações de serviços básicos urbanos, coleta de lixo, pavimentação e drenagem pluvial, podem ser fundamentais na redução da carga do patógeno e da transmissão. Neste estudo, propormos um protocolo de avaliação de intervenções governamentais de esgotamento com participação social (**capítulo três**). Este modelo de intervenções coparticipativas em saneamento, ou seja, integrar os tomadores de decisão com a comunidade local poderá contribuir para efetividade na redução de doenças ambientais multifatoriais. Adicionalmente, avaliamos como as restrições provocadas pela pandemia de COVID-19 afetou a circulação de reservatórios zoonóticos, ratos, dentro de uma comunidade urbana de Salvador-BA (**capítulo quatro**). Constatamos um aumento no avistamento de ratos durante o período pandêmico, o que pode representar um risco de leptospirose, reforçando a necessidade ações urgentes de controle do reservatório e da doença nessas comunidades. Por fim, sugerimos intervenções abrangentes e integradas de saneamento, estas ações podem contribuir para a lacuna de como estas ações podem impactar os mecanismos de transmissão e a carga de infecção por *Leptospira* nos residentes, assim como direcionar futuras intervenções com efeitos duradouros em outros locais no mundo.

Palavras-chaves: intervenção; esgoto; leptospirose; contaminação ambiental

SOUZA, F.N. Connecting rodents, environment, and humans of urban leptospirosis transmission dynamics: Effect of environmental and sanitation interventions on zoonotic diseases. 153pp. Thesis for the degree of Doctor of Ecology – Instituto de Biologia, Universidade Federal da Bahia, Salvador, 2023.

Abstract

Leptospirosis remains a serious health problem in impoverished urban communities in developing countries. The presence of polluted bodies of water and open sewage contributes to environmental contamination by the *Leptospira* bacteria and the abundance of rodents, representing the main source of exposure for residents in these locations. Ineffective rodent control programs and the absence of sanitary interventions contribute to the high incidence of Leptospira infection. Once sanitary and/or sewage interventions are implemented, their impacts should be evaluated to understand their effects on disease transmission, such as leptospirosis, and environmental health. This work is a study to evaluate the impact of government and community sanitary interventions on the environmental burden of *Leptospira*, the presence of rats, and *Leptospira* infection in the city of Salvador, Brazil. In the **first chapter**, we found that sewage intervention reduces rat refuge and movement sites, decreasing the likelihood of their presence in these areas. However, the intervention did not reduce the incidence rates of *Leptospira* infection, as residents' exposure did not vary between the periods before and after the intervention, limiting the understanding of the intervention's effect on disease burden. In **chapter two**, we show that government interventions to close sewage reduce the burden of *Leptospira* in the environment. The canalization and blocking of sewage and bodies of water prevent overflow during heavy rains, directing all water flow and reducing soil contamination. Integrated sanitary interventions, with local community involvement, in conjunction with other urban basic services such as garbage collection, paving, and stormwater drainage, may be crucial in reducing the pathogen burden and transmission. This study proposes an evaluation protocol for government sewage interventions with social participation (**chapter three**). This model of co-participatory sanitation interventions, i.e., integrating decision-makers with the local community, can contribute to the effectiveness of reducing multifactorial environmental diseases. Additionally, we evaluated how restrictions caused by the COVID-19 pandemic affected the circulation of zoonotic reservoirs, rats, within an urban community in Salvador-Ba (**chapter four**). We found an increase in rat sightings during the pandemic period, which may represent a risk of leptospirosis, reinforcing the need for urgent actions to control the reservoir and the disease in these communities. Finally, we suggest comprehensive and integrated sanitation interventions; these actions can contribute to addressing the gap of how these actions can impact transmission mechanisms and the burden of *Leptospira* infection in residents, as well as guide future interventions with long-lasting effects in other locations around the world.

Keywords: intervention; sewage; Leptospirosis; environmental contamination

SUMÁRIO

ESTRUTURA DA TESE

A presente tese está estruturada em quatro capítulos como segue:

Capítulo I- Explorando o efeito de uma intervenção em esgotamento sanitário sobre a infecção por *Leptospira* em comunidade de Salvador, Brasil

O capítulo teve como objetivo analisar o impacto de uma intervenção governamental de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos na presença dos roedores e na redução da incidência de infecção por *Leptospira* nos residentes de uma área desfavorecida de Salvador/BA. Para isso, utilizou-se um conjunto de dados longitudinais de monitoramento de roedores e coorte sorológica humana antes e depois da intervenção governamental. Diante disso, discutimos como intervenções baseadas em único componente do saneamento, canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos, pode impactar a transmissão de *Leptospira* e quais estratégias adicionais podem ser utilizadas para obtermos efeitos significativos na saúde humana e ambiental. Manuscrito a ser submetido ao periódico *Plos ONE*.

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

Este estudo tem como objetivo avaliar o papel dos esgotos na concentração e presença de *Leptospira* patogênica em amostras de solos de cinco comunidades urbanas de Salvador-BA, com diferentes sistemas de fechamento de esgotos (governamentais e comunitários). Este trabalho foi realizado em colaboração com Dr. Arnau Casanovas-Massana (Yale University) com o qual compartilho a autoria principal. Este trabalho foi publicado na *Environmental Science & Technology*.

Capítulo III- Simplified sewerage to prevent urban leptospirosis transmission: a cluster non-randomized controlled trial protocol in disadvantaged urban communities of Salvador, Brazil

Neste capítulo, propomos um protocolo de avaliação longitudinal de intervenções governamentais em esgotos com engajamento e participação comunitária, a fim de compreender os impactos destas intervenções co-participativas na incidência e nos mecanismos de transmissão da infecção por *Leptospira* e outras doenças. Este protocolo será aplicado em diferentes comunidades de Salvador em parceria com a Empresa Baiana de Águas e Saneamento (EMBASA), empresa gestora e executora das intervenções a serem avaliadas. Este protocolo foi submetido, em maio de 2022, na *BMJ Open journal.*

Capítulo IV- Increased rat sightings in urban slums during the COVID-19 pandemic and the risk for rat-borne zoonoses

Neste trabalho, discutimos como as restrições provocadas pela pandemia de COVID-19 acarretou mudanças comportamentais humanas gerando efeitos não intencionais na população de roedores nas comunidades urbanas, com precariedade de infraestrutura e serviços básicos de saneamento. Avaliamos longitudinalmente o número de avistamento de roedores por residentes em uma comunidade de Salvador-Bahia em diferentes períodos, antes e durante a pandemia por COVID-19. O resultado encontrado foi publicado no periódico *Vector borne and zoonotic diseases*.

INTRODUÇÃO GERAL

A leptospirose, doença causada por uma bactéria do gênero *Leptospira*, continua sendo uma zoonose emergente e de ampla distribuição em todo mundo (Bharti et al., 2003; Haake & Levett, 2015). No mundo são registrados mais de um milhão de casos, com 58.000 de mortes anuais (Costa et al., 2015; Hagan et al., 2016). A letalidade varia de 15% a 70% (Bharti et al., 2003; Karpagam & Ganesh, 2020). A maior incidência da doença se concentra, principalmente, nos países tropicais e em desenvolvimento urbano e socioeconômico, caso do Brasil. As infecções causadas pela bactéria *Leptospira* variam de formas assintomáticas a um estado febril, autolimitado e inespecífico, podendo também apresentar manifestações clínicas mais graves de infecção de múltiplos órgãos que pode ser relacionado à alta letalidade (Haake & Levett, 2015; Ko et al., 2009). A transmissão da leptospirose dá-se do animal reservatório da bactéria para humanos (contato direto com o sangue e urina de animais portadores da bactéria), mas também pode estabelecer-se de forma indireta pela transmissão ambiental (água e solo), ou seja, do reservatório ambiental para humano (contato com ambiente contaminado pela urina dos animais reservatórios) (Bierque et al., 2020; Ko et al., 2009; Minter et al., 2019). Por isso, além de ser uma zoonose a leptospirose pode ser considerada uma doença ambientalmente transmissível, já que das múltiplas formas de exposição e risco de contaminação para esta doença, o ambiente é modulador dos mecanismos de transmissão (Gerba, 2015; Hassell et al., 2017; Khalil et al., 2021).

A ecologia da leptospirose é complexa, visto que os mecanismos de transmissão podem variar em pequenas escalas, em consequência das interações da trinca de transmissão: animal reservatório, humanos e o ambiente (Eyre et al., 2022). Diversos animais podem albergar a bactéria *Leptospira*, como, por exemplo, porcos, gado, cães, ovelhas e cabras (Bharti et al., 2003) estes em especial, nos ambientes rurais. No entanto, dentro do contexto urbano, são ratos (principalmente *Rattus norvegicus*) o principal mantenedor da bactéria (Costa, Wunder, et al., 2015; Minter et al., 2019) e, a elevada abundância dos ratos está associada ao maior risco de infecção em humanos (Maciel et al., 2008; Minter et al., 2018). Condições urbanas inadequadas, como a presença de resíduos sólidos (lixo), alimentos e esgotos a céu aberto favorecem a sobrevivência, proliferação e probabilidade de presença destes animais, que coexistem em proximidade com as populações humanos (Feng & Himsworth, 2014; Panti-May et al., 2016). Além disso, a distribuição e movimentação dos roedores contribuem para espalhamento da bactéria no ambiente, principalmente em áreas urbanas mais baixas, regiões de escoamento das águas pluviais e propensos a eventos de alagamentos (Byers et al., 2019;

Eyre et al., 2022; Santos et al., 2017). Diante disso, intervenções de controle de roedores, como aplicação de rodenticidas têm sido ineficazes e o manejo de resíduos sólidos e esgotamento sanitário ainda são insuficientes nos grandes centros urbanos, contribuindo para estabelecimento e onipresença constante das populações de roedores (de Masi et al., 2009; Pertile et al., 2022; Santos et al., 2017).

Populações humanos residentes em comunidades urbanas vulnerabilizadas e empobrecidas, comumente denominadas de favelas, com ausência ou precárias condições de saneamento básico, sofrem com maior exposição a leptospirose e outras doenças infecciosas já descritas na literatura, caso da Dengue, Zika (Kikuti et al., 2015; Snyder et al., 2017) e recentemente, da covid 19 (SARS-CoV-2) que teve rápida disseminação nestas comunidades causando milhões de mortes, perdas econômicas e ampliação das iniquidades sociais e ambientais (Corburn et al., 2020; Fofana et al., 2022; Pereira et al., 2020). Soma-se outros efeitos indiretos como o aumento nos números de avistamento de ratazanas podendo apresentar um risco de coinfecção com leptospirose, somado aos casos de infecção por covid-19 (Amin et al., 2022; Kamath et al., 2022; Kiyokawa et al., 2022; Parsons et al., 2021).

Uma vez que, em contexto socioecológico, atributos sociais como, por exemplo, menor renda, baixa escolaridade e menor nível de conhecimento e percepções sobre a doença podem aumentar o risco para leptospirose (Palma et al., 2022; Ricardo et al., 2018). A proximidade dos domicílios ao esgoto a céu-aberto e a frequência de contato com o solo e água contaminados contribuem para elevada incidência nestes locais (Bierque et al., 2020; Khalil et al., 2021; Reis et al., 2008). Eventos climáticos, como chuvas intensas, contribui para o crescimento no número de casos de leptospiroses dentro destas comunidades(Hacker et al., 2020; Lau et al., 2010), dado que as chuvas podem aumentar a presença do patógeno no ambiente e amplia a chance de exposição à lama, solo e água contaminados devidos às deficiências na infraestrutura de manejo e drenagem das águas pluviais e esgotos sanitários (Bierque et al., 2020; Casanovas-Massana et al., 2018). Deste modo, observamos que a presença de esgotos a céu aberto é o fator-chave na dinâmica de transmissão da leptospirose urbana, posto que contribuem para presença de ratos, contaminação ambiental e consequentemente para exposição humana (Khalil et al., 2021; Mwachui et al., 2015).

O crescimento desordenado e as iniquidades sociais dentro dos espaços urbanos, no mundo, fazem com que 1,6 bilhões de pessoas vivam em condições inadequadas de infraestrutura e moradia, dos quais um bilhão residem em favelas ou assentamentos (Corburn et al., 2020). No Brasil, 15% da população vive nestes locais de elevada precariedade e negligência nos serviços urbanos básicos, como a ausência de saneamento (abastecimento de água potável, esgotamento sanitário, limpeza urbana e manejo de resíduos sólidos, drenagem e manejo de águas pluviais urbanas)(Andres et al., 2018). Apesar do saneamento apresentar-se na agenda global para desenvolvimento sustentável e do crescente número de países com acesso aos serviços de saneamento, ainda existem poucos investimentos para implementação e gerenciamento destes serviços (Dawes, 2022). Ações governamentais para melhoria de infraestrutura sanitária são caras e imprevisíveis, no entanto, quando implementadas, o impacto dessas intervenções deve ser avaliado na perspectiva da saúde ambiental e na saúde humana (Sokolow et al., 2019, 2022). A descentralização dos serviços públicos do saneamento dos outros serviços urbanos (limpeza urbana e drenagem), dificulta linhas orçamentarias destinadas para a melhorias sanitárias em comunidades vulnerabilizadas. O aumento dos investimentos e as políticas e programas voltados para essas populações, aliada à destinação adequada dos esgotos sanitários, podem ser uma alternativa eficaz para aumentar a resiliência do espaço urbano e reduzir o risco de doenças (Costa et al., 2017; Eyre et al., 2022). A implantação de soluções para os esgotos sanitários propicia mudanças na paisagem e outros efeitos não-intencionais, como, por exemplo, alterações comportamentais que podem alterar o ciclo de transmissão de doenças. Essas mudanças ambientais e de práticas de higiene podem impactar negativamente a densidade dos patógenos e vetores, reduzindo as chances de transmissão de doenças para população humana. No caso da leptospirose, com o esgotamento sanitário espera-se que diminua a distribuição e concentração da carga de *Leptospira* no ambiente e reduza a proximidade humana com o ambiente contaminado, assim como roedores.

O impacto de intervenções governamentais ou comunitários de esgotamento sanitário é amplamente reconhecida por reduzir a incidência de doenças virais, bacterianas e parasitárias (Andres et al., 2018), como exemplo na eficácia na prevenção a doenças diarreicas (Barreto et al., 2007). No entanto, seus possíveis efeitos na incidência da leptospirose, assim como na redução da contaminação patogênica por *Leptospira* no ambiente e na abundância dos roedores foram poucos examinados. Poucos estudos avaliaram as vias causais pelas quais o esgotamento sanitário pode reduzir as vias de infecções em países de baixa renda, sendo que essa avaliação é fundamental para subsidiar intervenções integradas de saúde pública em larga escala destinadas a reduzir a carga da leptospirose nessas comunidades urbanas negligenciadas.

A presente tese visa fornecer um conjunto de informações na perspectiva do avanço da compreensão sobre os efeitos de intervenções sanitárias governamentais ou comunitárias de destinação adequada dos esgotos sanitários na transmissão da leptospirose em ambientes urbanos. Além disso, notificar como mudanças naturais de caráter comportamental, por exemplo, o aparecimento SARS-CoV-2 afetou as populações de roedores nos espaços urbanos, podendo aumentar potencialmente o risco de transmissão da leptospirose e outras doenças. Os resultados destes estudos poderão ajudar na compreensão dos efeitos das mudanças ambientais, das obras de esgotamento sanitário, no risco zoonótico e, especificamente, na eco-epidemiologia e controle da leptospirose. Para mais, estes resultados poderão informar os esforços de vigilância e prevenção da leptospirose urbana e outras doenças, bem como destacar futuras oportunidades de pesquisa e subsidiar políticas de implementação de intervenções para o controle de doenças zoonóticas.

OBJETIVOS

Objetivo Geral:

Investigar os efeitos de intervenções sanitárias de esgotamento na dinâmica de transmissão de *Leptospira* sp por meio da avaliação dos três componentes envolvidos nos mecanismos de transmissão: 1) presença de roedores, 2) incidência de infecção em humanos e 3) concentração e presença do patógeno no ambiente.

Objetivos específicos:

- Avaliar o impacto do esgotamento sanitário na probabilidade de presença de roedores e na redução da incidência de infecção por *Leptospira* sp em humanos.
- Determinar o efeito dos esgotos sanitários na contaminação ambiental por *Leptospira* patogênica.
- Elaborar um protocolo para avaliar a efetividade de intervenções em esgotamento sanitário com engajamento comunitário na redução de incidência de leptospirose e os efeitos nos mecanismos de transmissão.

Adicionalmente,

- Caracterizar os efeitos de mudanças comportamentais ocasionadas pela pandemia de COVID-19 na população de roedores, principal reservatório de *Leptospira* sp, em comunidades urbanas empobrecidas.

METODOLOGIA GERAL

<u>Área de Estudo</u>

O município de Salvador/BA possui população estimada de mais de dois milhões de habitantes e 41,8% (882.204 hab.) dos domicílios estão concentrados em favelas ou aglomerados subnormais conforme o Censo Demográfico de 2019 (IBGE, 2020). Em vista disso, cerca de 12% (345.000 mil habitantes) da população vivem em locais sem acesso ao esgoto ou com esgotamento precário, manejo de resíduos sólidos irregular e em domicílios sujeito a alagamentos ou inundações (IBGE, 2020). A vulnerabilidade e o gradiente socioambiental das metrópoles, como Salvador, faz com que a população residente destes espaços esteja exposta a diferentes patógenos zoonóticos e ambientais como, por exemplo, Dengue, Zika, leptospirose e recentemente a Covid 19 (Sars-Cov2), em elevada incidência e mortalidade. No que tange à doença leptospirose, as comunidades carentes de Salvador apresentam alta incidência, 58,7 casos por 100.000 hab. (Hagan et al., 2016). Neste contexto, nesta tese foram estudadas cinco comunidades de baixas condições de moradia e precariedade no saneamento na cidade de Salvador-Bahia, a saber: Pau da Lima, Nova Constituinte, Sete de abril, Tancredo Neves e Campinas de Pirajá (**Figura 1**).

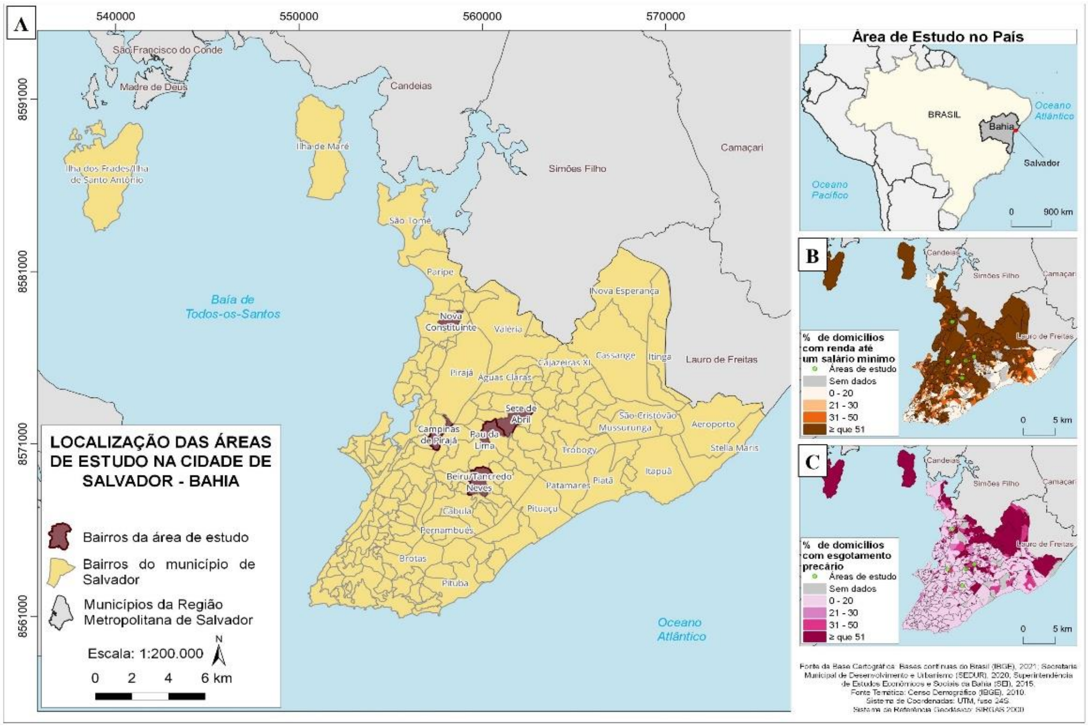

Figura 1. Território e regiões administrativas da cidade de Salvador-Bahia-Brasil. Em **A**, as cincos comunidades urbanas envolvidas no estudo. (**B**) Distribuição socioeconômica a partir da proporção de renda por domicílio. (**C**) Proporção de domicílios com saneamento precário.

Intervenção sanitária

As intervenções sanitárias incorporadas neste estudo foram aquelas que visaram ampliar a rede coletora de esgotos sanitários nas comunidades urbanas de condições socioeconômicas baixas. As intervenções realizadas nas comunidades buscaram reduzir o despejo de esgotos sanitários diretamente na rua ou em córregos, rios ou em redes de águas pluviais, e ainda ampliar as ligações entre a rede coletora e o domicílio. As ações foram executadas por instituições governamentais de infraestrutura e saneamento, incluindo obras de drenagem e canalização dos corpos d'água poluídos com esgotos domésticos. As obras foram realizadas pela Empresa Baiana de Águas e Saneamento (EMBASA), Secretaria Municipal de Infraestrutura e Obras Pública (SEINFRA-Salvador) e/ou Companhia de Desenvolvimento Urbano do Estado da Bahia (CONDER). As intervenções sanitárias governamentais de esgotamento sanitário realizadas pela Embasa, envolveram a implantação de rede coletora do tipo convencional e condominial, que recolheram os esgotos domésticos dos domicílios das áreas de estudo (Melo, 2008). A rede condominial de esgotos dispõe da rede básica e ramais condominiais. Nos ramais condominiais faz-se a coleta dos esgotos domésticos de um conjunto de casas, interligando casa a casa, para depois os esgotos serem lançados na rede básica (Melo, 2008). Além disso, incluímos intervenções realizadas pela comunidade local, aqui chamadas de intervenções comunitárias, estas intervenções consistiram no fechamento do esgoto a céu aberto com placas de concretos e/ou madeiras que podem servir como uma barreira de proteção para contato com o esgoto. Áreas controles foram adicionadas ao estudo, são áreas com trechos de esgotos a céu aberto e baixa infraestrutura de drenagem.

Coleta de dados

Os dados utilizados neste estudo são oriundos de uma pesquisa eco-epidemiológica de longa duração de base comunitária, que através de um estudo de coorte prevê compreender a história natural da leptospirose urbana e outras doenças (Grants: R01 AI052473, U01AI088752, R01 TW009504, R25 TW009338-*National Institutes of Health*; 102330/Z/13/Z, 218987/Z/19/Z-*Wellcome Trust* e JCB0020/2016- *Fundação de Amparo à Pesquisa do Estado da Bahia*).

Todos os métodos e análises dos dados utilizados estão detalhadamente descritos em capítulos específicos desta tese.

De maneira geral, recrutamos e acompanhamos 1.058 moradores de uma comunidade urbana de Salvador/BA, bairro de Pau da Lima, durante nove períodos sazonais entre 2015-2022. Especificamente, identificamos a presença de anticorpos anti-*Leptospira* nos residentes por meio do teste de aglutinação microscópica (MAT) (Reis et al., 2008). Entrevistas estruturadas foram realizadas, incluindo informações sobre as características individuais do participante, exposições previas e o contato e avistamento de roedores no domicílio ou peridomicílio. Todos os procedimentos de consentimento informado foram aprovados pelo Conselhos de Ética em Pesquisa da Fundação Oswaldo Cruz e Comissão Nacional de Ética em Pesquisa do Brasil, Ministério da Saúde do Brasil (CAAE: 01877912.8.0000.0040). Os dados de presença de roedores foram obtidos através das marcas e sinais deixados nas placas de rastreamentos. As placas de rastreamento é um método validado como um substituto para a quantificar a infestação de roedores (Eyre et al., 2020; Hacker et al., 2016).

Além disso, para compreender o impacto das intervenções na contaminação ambiental em diferentes comunidades de Salvador/BA, amostras de solos foram coletadas em diferentes áreas, com e sem esgotamento sanitário, para detecção da presença e concentração de *Leptospira* por Reação em Cadeia da Polimerase quantitativa (qPCR) (Casanovas-Massana et al., 2018).

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Capítulo I

Artigo a ser submetido no periódico *Plos one*

Explorando o efeito de uma intervenção em esgotamento sanitário sobre a infecção por *Leptospira* em comunidade desfavorecida de Salvador, Brasil.

(*Exploring the effect of an intervention in urban sewerage on Leptospira infection in a disadvantaged community in Salvador, Brazil*)

Título: Explorando o efeito de uma intervenção em esgotamento sanitário sobre a infecção por *Leptospira* em residentes de comunidade desfavorecida de Salvador, Brasil

English title: Exploring the effect of an intervention in urban sewerage on *Leptospira* infection in a disadvantaged community in Salvador, Brazil

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A leptospirose é uma zoonose que afeta comunidades carentes em todo o mundo. Nestas comunidades, o esgoto a céu aberto tem se mostrado um importante fator de risco associado à transmissão da leptospirose. No entanto, o papel de intervenções que melhoram condições de esgotamento sanitário na redução da transmissão da *Leptospira* ainda não foi avaliado. O objetivo deste estudo foi avaliar a eficácia de uma intervenção de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos realizada pelo governo local em duas áreas de uma comunidade urbana de Salvador-BA. O estudo utilizou modelos estatísticos de medidas repetidas para avaliar a efetividade desta intervenção nas populações de ratos e nas infecções individuais por *Leptospira* identificadas nos residentes através dos testes de anticorpos de microaglutinação (MAT). O estudo de coorte, em andamento, realizou soroinquéritos semestrais de agosto de 2015 a agosto de 2022 na comunidade de Pau da Lima em Salvador, Brasil. As intervenções foram realizadas em 2018 e 2021 pelo governo em duas áreas desta comunidade. Selecionamos uma área controle para cada período para avaliar a eficácia da intervenção. Para avaliar o efeito da intervenção na população de roedores utilizamos as placas de rastreamentos com proxies de abundância e atividades dos ratos em sete períodos sazonais. Embora a intervenção tenha reduzido a presença de roedores (OR: 0,40 CI 95% 0,33-0,49), não houve redução nas taxas de infecção por Leptospira nos residentes das áreas de intervenção. Inesperadamente, encontramos um aumento nas chances de infecção nas áreas de intervenção de 2,53 (CI 95% 1,42, 4,69) na primeira área e 2,61 (95 % CI 1,37, 4,74) na segunda área durante o período de estudo. Além disso, não houve diferença significativa observada entre as chances de infecção por *Leptospira* no controle ao longo do tempo e quando as áreas de controle e intervenção que foram comparadas após a intervenção. Nossos achados sugerem que intervenções focadas apenas na canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos não interrompem todas as vias de transmissão da leptospirose. Mesmo após as obras, os moradores permaneceram frequentemente expostos a alagamentos, lama e solo, o que contribuiu para a manutenção dos altos índices de infecção por *Leptospira*. Para abordar efetivamente as intervenções em áreas de alta exposição, as intervenções devem ser projetadas e implementadas considerando múltiplas dimensões do esgotamento sanitário. Tais estratégias podem garantir a sustentabilidade e melhorar a qualidade ambiental, levando à redução da incidência de doenças.

Palavras-chave: Leptospirose, roedores, intervenção sanitária.

Introdução

O acesso a instalações sanitárias adequadas ainda é um problema crítico nos grandes centros urbanos de diversos países, fazendo com que mais de um bilhões de pessoas vivam em locais insalubres, com carência de serviços básicos, como, por exemplo, a coleta de resíduos sólidos, esgotamento sanitário, drenagem das águas pluviais e melhorias na infraestrutura habitacional (UN-Habitat, 2022). Estas deficiências sanitárias estão associadas positivamente ao risco de doenças infecciosas zoonóticas de cunho ambiental (Rees et al., 2021), e, por consequência, melhorias no sistema de esgoto podem impactar na redução da carga destas doenças e na qualidade de vida das pessoas (Bradley & Altizer, 2007; Eskew & Olival, 2018; Goarant, 2016). A leptospirose, é uma doença de elevada mortalidade e morbidade nos países em desenvolvimento, sendo uma enfermidade associada a pobreza e a precariedade dos serviços de saneamento básico, com destaque para o esgotamento sanitário e drenagem das águas pluviais urbanas, além da higiene doméstica. (Bharti et al., 2003; Khalil et al., 2021; Lau et al., 2010).

A infecção por bactérias patogênicas do gênero *Leptospira* dar-se pelo contato com água ou solo contaminado pela urina dos roedores infectados, principalmente *Rattus norvegicus*, considerado o principal reservatório animal nos ambientes urbanos (Haake & Levett, 2015; Karpagam & Ganesh, 2020; Minter et al., 2018). No mundo, são registrados mais de um milhão de casos de leptospirose e uma carga anual de 58 mil mortes, principalmente após eventos de chuvas fortes e inundações, em especial nos países em desenvolvimento (Costa et al., 2015). Estudos em comunidades urbanas desfavorecidas, ou favelas, no Brasil mostraram forte associação entre a presença de esgotos a céu aberto com a abundância de roedores e risco de transmissão por *Leptospira* em residentes (Costa et al., 2017; Khalil et al., 2021; Minter et al., 2019). A presença de esgotos a céu abertos e de canais pluviais com despejos domésticos funcionam como mantenedores da contaminação ambiental, uma vez que contribui para a sobrevivência e dispersão das leptospiras que foram excretados pelos ratos, acentuando-se durante a estação chuvosa onde há intensificação do escoamento de águas pluviais e transbordamento dos esgotos (Bierque et al., 2020; Casanovas-Massana et al., 2018; Guo et al., 2022). Adicionalmente, as águas de córregos, mesmo poluídos por esgotos domésticos, são fontes de água para os roedores, e as redes de drenagem ou de esgotamento sanitário contribuem para refúgio e movimentação desses animais e, consequentemente, favorece maior risco de infecção humana devido à proximidade dos residentes a estes reservatórios animais. As estratégias atuais de prevenção da leptospirose têm sido ineficientes tanto na redução dos reservatórios, quanto na diminuição no número de casos da doença (Bharti et al., 2003; Colvin, 2002; De Masi et al., 2009b), devido principalmente a multifatoriedade desta doença (Mwachui et al., 2015). Muitos pesquisadores vêm sugerindo intervenções sanitárias e ambientais que visem no sistemas de esgoto adequados, com estruturas de drenagem construídas para o escoamento das águas de chuva e córregos que recebem e transportam também esgotos domésticos, podendo proteger os residentes da contaminação (Costa et al., 2017; Khalil et al., 2021; Minter et al., 2019).

Expansão e melhorias na cobertura de esgoto urbanos mostrou-se eficaz na redução de doenças e na qualidade ambiental em muitos lugares, como o caso das doenças diarreicas (Barreto et al., 2007; Genser et al., 2008). No entanto, sua implementação e ampliação ainda não é acessível, principalmente devido ao alto custo, dificuldades logísticas e grandes extensões de áreas que necessitam de intervenções urgentes (Andres et al., 2018; Scott et al., 2019). Uma vez implementadas, devem ser caracterizados e avaliados seus impactos na saúde e a sustentabilidade das obras, porém, o tempo necessário para a produção desses impactos do pós-obra e replicabilidade de implementação dificultam estas avaliações. Estes resultados possibilitarão definir novas configurações de intervenções em outras áreas urbanas carentes, como tomada de decisão de soluções adequadas às realidades locais, contribuindo para o cumprimento das metas dispostas nas agências governamentais locais e mundiais (Sustainable Development Goal, 2015; Yamey et al., 2014) .

A quarta maior cidade metrópole do Brasil, Salvador, apresenta uma população de 2,9 milhões de habitantes, sendo que cerca de 345 mil pessoas vivem em locais com esgotos a céu aberto, sem serviços urbanos básicos e com deficiências significativas na coleta dos resíduos sólidos (IBGE, 2020). Esta condição faz com que Salvador, apresente uma elevada abundância de roedores, coexistindo em proximidade com os residentes, e uma alta incidência de leptospirose, 58,7 casos por 100.000 hab. no município (Eyre et al., 2022; Minter et al., 2019; Panti-May et al., 2016). Entre os anos de 2018-2019, o poder público local executou obras de canalização e tamponamento dos córregos pluviais contaminados com esgotos domésticos, comumente relatados de forma genérica de esgotos, em um distrito sanitário de saúde do município, gerando uma oportunidade inédita para medir o impacto desta intervenção de esgotamento sanitário na infecção por *Leptospira* sp, possibilitando, explorar como intervenções especificas de fechamentos dos esgotos atuam na saúde ambiental e humana.

Para tanto, o objetivo deste estudo foi explorar o impacto de uma intervenção de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos na infecção por *Leptospira* em um ambiente de comunidades carentes em Salvador, Brasil. Especificamente, i) avaliamos a efetividade na redução da atividade dos roedores e ii) na incidência de transmissão de *Leptospira* na população residente.

Métodos

Descrição da intervenção

O estudo avaliou obras de intervenções sanitárias realizadas pela Secretaria Municipal de Infraestrutura e Obras Pública (SEINFRA-Salvador), empresa pública de infraestrutura e saneamento de esfera municipal. As intervenções foram realizadas na comunidade urbana de Pau da Lima em Salvador/BA (**Fig. 1A**). Previamente descrita, a comunidade de Pau da Lima possui aproximadamente 3717 habitantes residindo em uma área de 0,25km^2 divididos em três vales interconectados (vale 1,2 e 3) (Eyre et al., 2022; Hagan et al., 2016). O local é caracterizado por um gradiente de elevação topográfica e desigualdades socioeconômicas, no qual as áreas mais baixas de cada vale apresenta de esgotos a céu aberto, lixo e péssimas condições de moradia que expõem os residentes a diferentes riscos de doenças. Estes fatores contribuem para elevada incidência anual de infecção por *Leptospira*, 37,8 por 1000 habitantes (Hagan et al., 2016) e para abundância constante dos ratos próximos aos domicílios (Panti-May et al., 2016).

As obras compreenderam na canalização e tamponamento de córregos poluídos, que historicamente têm transportado esgotos domésticos lançados diretamente nesses corpos d'água e melhorias na rede coletora, conectando os domicílios à rede de esgoto interceptora (**Fig. 2**). As intervenções foram realizadas em duas áreas de aproximadamente 0,3 km^2 (vale 2 e 3), em dois períodos distintos (2018 e 2020) (**Fig. 1C**). O vale 1 foi utilizado neste estudo como controle, região sem intervenções. Observamos com os resultados da obra, de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos, aumento das superfícies pavimentadas e construções de vias de acesso, como por exemplo escadas e ruas (**Fig. 2**).

Fonte dos dados

Utilizamos um conjunto de dados oriundos de estudo de base comunitária de longa duração, em andamento, que se objetivou compreender a história natural da leptospirose urbana no município de Salvador/BA (De Araújo et al., 2013; Hagan et al., 2016). O estudo foi aprovado pelos Conselhos de Ética em Pesquisa do Instituto Gonçalo Moniz, da Fundação Oswaldo Cruz (Fiocruz) e pela Comissão Nacional de Ética em Pesquisa (CAAE 17963519.0.0000.0040). Além deste conjunto de dados, utilizamos dados de monitoramento de roedores nos espaços de comunidades urbanas em vulnerabilidades ambiental e social (Eyre et al., 2020; Hacker et al., 2016). Todos as etapas e processamento dos dados estão descritos nos tópicos a seguir.

Identificação da infecção por Leptospira

Foi realizado uma coorte prospectiva estacionais de 08/2015 a 08/2022, para identificar infecções assintomáticas por *Leptospira*. Um total de dez acompanhamentos foram realizados, sendo um de linha de base seguido por nove acompanhamentos semestrais, que foram utilizados para calcularmos a soroincidência entre os períodos (**Fig. 1C**). Todos os critérios de inclusão dos participantes envolvidos neste estudo foram similares aos descritos em estudos anteriores (Eyre et al., 2022; Hagan et al., 2016). De todos os participantes recrutados e consentes, foram coletadas amostras de sangue por uma equipe técnica habilitada. Durante a coleta foram realizadas entrevistas individuais, sendo coletadas informações sociodemográficas, características do ambiente domiciliares e exposições a fontes potenciais de contaminação ambiental nos últimos seis meses que antecederam as coletas. Dentre as informações coletadas estão: contato com fontes de água (chuva, esgoto e inundação), alagamento domiciliar, percepções de riscos e vulnerabilidades, avistamento de ratos, presença de lixo e práticas comportamentais que possam reduzir a exposição a bactéria no ambiente.

A presença de anticorpos anti-*Leptospira* patogênica nas amostras de soro obtidas na coleta de sangue dos participantes, foram identificados utilizando teste de microaglutinação (MAT) conforme descrito por Goris & Hartskeerl, 2014 (Goris & Hartskeerl, 2014). A infecção foi definida pela soroconversão entre amostras negativas para uma outra com diluição de títulos ≥1:50 ou um aumento de quatro vezes no título entre as amostras consecutivas entres os participantes da coorte (Cruz et al., 2022; Eyre et al., 2022). Exploramos também, o decaimento de títulos de anticorpos anti-*Leptospira* medidos entres os períodos de acompanhamento semestrais (Bonner et al., 2021).

Monitoramento dos roedores

Para quantificar a infestação dos roedores, realizamos sete campanhas sazonais, intercalando-se entre os acompanhamentos sorológicos, nos períodos de 04/2016 a 02/2020. A infestação de roedores foi medida através de um *proxy* de abundância oriundo das placas de rastreamento (Hacker et al., 2016). A placas rastreamento são folhas de poliestireno (acetato) de dimensões 0,2 × 0,2m, pintadas com *Lampblack* que permite a detecção de marcas especificas deixadas pelos roedores, como por exemplo, arranhões, pegadas e caudas. Um total de 415 pontos foram randomizados e em cada ponto foram instalada um conjunto de cincos placas dentro de um raio de 5m durante dois dias consecutivos. A cada 24h, estas placas foram fotografadas e analisadas quanto a presença de roedores em pelo ao menos uma placa de cada ponto. A presença de ratos foi definida pela proporção de placas com atividades de roedores em cada localidade amostral.

Análises dos dados

Primeiro, utilizamos a estrutura do modelo multivariado de efeitos mistos para avaliar o efeito da intervenção sanitária, definido como uma variável de presença e ausência de intervenção (canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos), na atividade dos roedores. A presença de ratos em cada unidade amostral incluiu uma proporção de placas com marcas de ratos controladas por covariáveis preditoras ambientais já descritas em outros estudos, como presença de lixo, proximidade com esgoto, presença de vegetação (alta e irregular) (Himsworth et al., 2014; Rothenburger et al., 2017; Tamayo-Uria et al., 2014). As unidades amostrais foram inclusas no modelo como um fator randômico. Neste modelo apenas incluímos a intervenção ocorrida no vale 2, pois não tivemos amostragens de roedores nos períodos que ocorreram as intervenções no vale 3 (**Fig 1C**.).

Para os dados humanos, a partir de um desenho de coorte pareada, ou seja, com controles concorrentes, calculamos as taxas de incidência de infecção por *Leptospira* nas áreas controle (vale 1) e de intervenção (vale 2 e 3), antes e após a intervenção. Avaliamos as diferenças proporcionais entre as incidências antes e após a intervenção em momentos específicos. Ao final, o risco atribuído às deficiências do esgotamento sanitário foram determinadas para cada período após a intervenção comparados ao sítio controle.

Equações de estimativa generalizada (GEEs) (Huang et al., 2016) foram calculadas para investigar se as intervenções sanitárias de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos realizadas no vale 2 e 3 tiveram efeito sobre a incidência de infecção por *Leptospira*, nos diferentes períodos (antes, durante e após a intervenção sanitária). Dois modelos independentes foram calculados, o modelo principal inclui-se o aumento de quatro vezes na titulação e a seroconversão como variável resposta e um segundo modelo utilizamos como resposta principal o decaimento da titulação. Os modelos lineares generalizados marginais, assumem uma correlação comum das medidas de respostas repetidas, uma vez que o objetivo desta análise é fazer inferências sobre a média da população, ao invés de respostas individuais (Huang et al., 2016; Zeger & Liang, 1986; Zhang et al., 2019). Modelamos o efeito das covariáveis e a associação entre observações de um participante em cada período. Para variáveis preditoras, variáveis de interação entres áreas (vale 1-controle e vales 2 e 3- áreas de intervenção) e tempo também foram incluídas no modelo, juntamente com as covariáveis demográficas (sexo e idade) e espacial (elevação). O nível de significância adotado foi de 0,05.

Todas as informações anonimizadas foram armazenadas e gerenciadas no sistema eletrônico de captura de dados REDCap (Harris et al., 2009). As análises estatísticas foram realizadas usando R (R Core Team and others, 2016), utilizando os seguintes pacotes: *geepack* (função *geeglm*) (Halekoh et al., 2006) e lme4 (Bates et al., 2015).

Resultados

Verificamos que a intervenção de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos, no vale 2, reduziu 60% a chance da presença dos ratos nas áreas domiciliares e peridomiciliares, (OR 0,40 (IC:0,33-0,49). No entanto, a presença de vegetação (OR 1,81 (IC:1,51-2,17) e acúmulo de lixo (OR 2,28 (IC: 2,03-2,57) contribuíram para um aumento de duas vezes na probabilidade de presença dos ratos. A presença dos ratos variou com a distância em relação aos esgotos (**Tabela 1**).

No que se refere à infecção por *Leptospira*, um total 1058 participantes foram acompanhados em nove períodos sorológicos, uma média de 193 participantes no vale 1, 436 no vale 2 e 429 no vale 3. As características individuais e o perfil sociodemográfico da população estudada já foram descritas em trabalhos anteriores (Eyre et al., 2022; Hagan et al., 2016). Observamos uma heterogeneidade nas taxas de incidências entre os períodos e vales estudados (**Fig. 3**).

A frequência dos comportamentos específicos (caminhar descalço e avistar de roedores próximo ao domicílio) e exposições de contato com águas pluviais, esgoto, chuva ou oriunda de inundações nos últimos seis meses anteriores às entrevistas foram quantificadas e apresentadas nas Figuras 4 e 5. Além disso, sobre as exposições de riscos domiciliares encontramos diferenças nas frequências de inundações de águas pluviais entres os vales nos diferentes períodos, mas não observamos diferenças nas taxas de frequência de alagamentos em domicílios e de presença de resíduos sólidos (lixo) próximo aos domicílios entre os vales 1, 2 e 3 (**Fig. 5**). Evidenciamos que, a percepção da seriedade e do grau de vulnerabilidade individual quanto a doença leptospirose foi de extremamente séria à risco alto em todos os períodos de acompanhamento (**Fig. Sup. 2**).

As incidências de infecção assintomática por *Leptospira* para as áreas com intervenção e seu controle simultâneos para cada área, antes e em diferentes períodos após a intervenção estão apresentados na Tabela 2. Nas áreas com intervenção, as taxas de incidência antes das obras foram 27,78 (IC:14,87-47,03) por 1000 habitantes (hab.) no vale 2 (período 3) e 30,73 (IC:16,46-51,98) por 1000 hab. para o vale 3 (período 6). Para a área controle (vale 1), ou seja, sem fechamentos dos esgotos, as incidências de infecção foram 60,87 (IC:33,67-100,02) e 80,57 (IC:47,63-125,87) casos por 1000 hab., se comparados, respectivamente, com os resultados dos vales 2 e 3, onde foram realizadas as intervenções. Oito meses após as intervenções no vale 2 (período 6), observamos uma taxa de incidência de 56,65 (IC:36,24-83,79) por 1000 hab., valor maior que a taxa de incidência para o mesmo período na área controle, representando um aumento de 71,56% comparado a incidência anterior à intervenção.

Avaliando outros momentos após as intervenções, encontramos aumento na incidência de infecção por *Leptospira* (**Tabela 2**). Dois anos após (período 7) da intervenção no vale 2, as taxas de incidência tiveram aumento de 36,03%, enquanto na área controle obtivemos uma redução de 33,39%. Evidenciamos também que, após dois anos e seis meses observamos uma redução de 11,63% na taxa de incidência comparados aos valores do controle, que teve um aumento de 51% na sua incidência. Em resumo, mostramos que o nosso controle apresentou uma taxa de incidência de 91,89 (CI:54,44-143,05) por 1000 hab. E, para o mesmo período, depois da intervenção, apresentou-se uma incidência de 24,55 (CI:12,31-43,50) por 1000 habitantes. Isso significa uma redução de risco atribuído a canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico de 62,59%. Contrariamente, os períodos seguintes observaram-se um aumento no risco atribuído e as taxas de incidências antes e depois da intervenção em ambas as áreas foram similares. Sobre a segunda intervenção, no vale 3, conseguimos obter apenas dois seguimentos após a intervenção. Primeiro, quatro meses após a intervenção observamos um aumento de 68.92% na taxa de incidência na área de intervenção e uma menor taxa na área controle (14,05%). No entanto, um ano após a intervenção a incidência na área controle e intervenção tiveram valores proporcionais inversos, respectivamente representou um aumento de 39,08% na área de intervenção, enquanto na área de controle uma redução de 34,04% na taxa de incidência.

No geral, considerando as médias das incidências entre os períodos, encontramos um aumento de 50,91% na incidência de infecção por *Leptospira* no grupo de intervenção e uma diminuição de 5,92% no grupo controle. Observamos então, que 56.83% do risco de infecção por *Leptospira* pode ser atribuída as deficiências das obras de canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico, que pode formar condições para a proliferação de ratos e áreas com maiores chances de inundação.

Nos modelos equações de estimativas generalizadas, no qual avaliamos a intervenção de forma longitudinal considerando a dependência entre as taxas de infecção individuais (**Tabela 3**), observamos que, para os indivíduos que tiveram aumento de quatro vezes na titulação ou sofreram soroconversão, a idade dos indivíduos afetou positivamente na chance de infecção nas duas áreas de intervenção. Assim como, o sexo biológico masculino, apresentou uma OR 1,51 (IC:1,10, 2,06) para a intervenção no vale 2 e OR 1,76 (IC:1,30, 2,36) para o vale 3. Participantes que residiam nas áreas mais altas do vale 2, elevação superior a 15 metros, obtiveram menor risco de infecção por *Leptospira* (OR 0.49 (IC:0,32, 0,76). No entanto, não observamos este efeito na intervenção no vale 3. Para ambas as intervenções, após a canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico observamos um aumento na chance de infecção por *Leptospira*, respectivamente OR 2,53 (IC:1,42, 4,69) - vale 2 e OR 2,61 (IC:1,37, 4,74) -vale 3. Não encontramos diferenças significativas e variação na positividade entre os diferentes momentos da área de intervenção e a controle (**Tabela 3 e Fig. Sup. 1**). Avaliando o decaimento nos títulos de anti-*Leptospira*, observamos resultados similares quanto à idade, sexo e a topografia local (elevação), e não observamos efeitos significativas da intervenção sobre a infecção por *Leptospira* (**Tabela suplementar 1**).

Discussão

Neste estudo observamos que intervenções de canalização e tamponamento de canal para conduzir águas pluviais, córregos e esgotos domésticos lançados inadequadamente, foram eficientes no controle de roedores, visto que melhorias na rede sanitária reduzem a probabilidade de presença de ratos nos ambientes urbanos carentes. Todavia, encontramos poucas evidências de que essa intervenção, canalização e tamponamentos dos canais pluviais poluídos, reduzem o risco de infecção por *Leptospira*, sendo que não observamos efeitos na incidência de infecção nos residentes da comunidade urbana carente de Pau da Lima, em Salvador-Bahia.

Conforme esperávamos, a intervenção afetou negativamente na atividade dos roedores, reduzindo em 60% na chance de presença nas áreas que ocorreram as intervenções de esgotamento. Este resultado, mais uma vez, reforçam os achados previamente descritos por Awoniyi e colaboradores (Awoniyi et al., 2022), que mostrou a efetividade do manejo químico aliada a obras de habitação e estruturais urbanas na redução da infestação de roedores na mesma comunidade estudada. Resultados similares foram já relatados nas Bahamas (Awoniyi et al., 2021), Singapura (Soh et al., 2023), Hungria (Bajomi D, 2013), Canada (Colvin et al., 1998) e no Brasil (Awoniyi et al., 2022; De Masi et al., 2009a; Pertile et al., 2022), mas nenhum destes estudos avaliaram o impacto exclusivo de intervenções de melhorias sanitárias na infestação dos roedores, o que dificulta comparações entre os resultados encontrados. As disparidades entre as intervenções realizadas no mundo geram lacunas sobre quais conjuntos de intervenções ótimas para controle de ratos, que apresentem durabilidade dos efeitos, menor grau de resiliência e que sejam operadas e mantidas adequadamente, principalmente nos ambientes urbanos carentes.

Dentro dos espaços urbanos densamente povoados, favelas, as melhorias no sistema de esgotamento e no manejo de águas pluviais facilitam o movimento dos roedores a longa distâncias o que pode ter contribuído para a diminuição da abundância relativa local. Apesar da importância de estas intervenções gerarem unidades de erradicação, afetando as populações de roedores locais, ainda não está claro como estas mudanças de paisagem contribuem para movimentação dos ratos para as áreas vizinhas (Byers et al., 2019; Richardson et al., 2017). Esse efeito significativo da canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico sobre a presença dos roedores nos espaços de comunidades carentes pode representar uma possível redução na carga de patógenos em proximidade dos canais pluviais poluídos canalizado, popularmente chamados de esgotos. Essa redução pode acarretar a diminuição de pontos quentes de contaminação ambiental dada distribuição e prevalência de *Leptospiras* patogênica. Estudos recentes, avaliando diferentes seções de esgotos a céu aberto e fechados, mostraram que prevalência de *Leptospira* é menor nos locais de esgotos fechados pelo poder público (Casanovas-Massana et al., 2021). Foi observado que a prevalência de *Leptospira* nos solos ao redor dos sistemas de esgoto governamental de Pau da Lima, Salvador, Brasil, foi de 8% comparados aos locais de esgoto a céu aberto que apresentou uma prevalência de 54% e elevada concentração do patógeno (Casanovas-Massana et al., 2021). Contudo, a intervenção de esgotamento age diminuindo a área de vida dos roedores e isola o ambiente da contaminação via os esgotos, principalmente durante eventos de chuvas fortes (Bierque et al., 2020).

Apesar dos significativos efeitos na população de ratos, a precariedade de serviços urbanos básicos como o manejo de resíduos sólidos (coleta de resíduos sólidos e retirada de vegetação alta) assegurou a manutenção constante das populações de roedores. Estes fatores de riscos, contribuem como fonte de alimento e refúgios, e a disponibilidade destes atributos favorece as flutuações temporais das densidades dos roedores urbanos (Panti-May et al., 2016). A presença de resíduos urbanos em proximidade das residências garante maior disponibilidade de alimento para os ratos, melhorando sua condição corporal, aumentando sua aptidão reprodutiva e capacidade de albergar a bactéria *Leptospira* e outros parasitas (Carvalho-Pereira et al., 2019). Associado a coleta irregular de lixo, a presença de vegetação também está associada significativamente com a abundância relativa dos roedores, estes micro-habitat na paisagem urbana proporcionam locais de nidificação e de interações intraespecíficas, reduzindo as taxas de predação (Cavia et al., 2009; Madden et al., 2019). Os fragmentos irregulares de vegetação, portanto, garantem a estabilidade das colônias dos roedores, o que pode ser uma via de risco de transmissão de patógenos dentro da população dos ratos, como, por exemplo, a bactéria *Leptospira* sp, o vírus SEOV e, recentemente SARScov2 presente na população de *Rattus novergicus* (Costa et al., 2014; Wang et al., 2023). Estes fatores associados à presença dos ratos podem ser maiores do que imaginávamos, o que pode minimizar ou confundir o impacto da intervenção de canalização dos córregos poluídos sobre o dinâmica de exposição dos residentes. Contudo, uma vez que o controle de ratos, com uso de rodenticidas, reduzem temporariamente as populações (De Masi et al., 2009a; Minter et al., 2019; Pertile et al., 2022)(Costa et al., 2017; Sokolow et al., 2019), as intervenções combinadas de infraestrutura de habitação associadas ao esgotamento sanitário tem sido a estratégia mais eficaz e duradoura no controle de roedores nos espaços urbanos carentes, limitando uma das vias de exposição a bactéria *Leptospira* pelos residentes (Costa et al., 2017; Sokolow et al., 2019).

No que se refere à infecção assintomática por *Leptospira*, os resultados do nosso estudo indicaram que as obras no sistema de esgoto, implementados pelo poder público na comunidade de Pau da Lima, na cidade de Salvador-Bahia, não tiveram efeitos na redução da incidência de infecção por *Leptospira*, apresentando um efeito inesperado, aumentando as taxas de incidência períodos após a intervenção. Todavia, temporalmente, observamos alguns efeitos positivos intermediários na redução do risco dos indivíduos associado com a esta intervenção sanitária.

Evidenciamos, que os residentes envolvidos no estudo apresentam uma frequência de exposição contínua aos fatores riscos associados à leptospirose, como contato a diferentes coleções de águas contaminadas (de chuva, esgoto etc.) e lama, ocasionando as elevadas taxas de incidência. A natureza multicausal da doença, com diferentes níveis hierárquicos, pode ter contribuído para não observamos a efetividade da intervenção (Karpagam & Ganesh, 2020). Isso porque, os fatores socioeconômicos como péssimas condições de moradia, baixo acesso aos serviços básicos urbanos, menor renda e educação, além das características topográficas, que impõe riscos desiguais em uma mesma área geográfica, contribui para alta ocorrência de leptospirose, minimizando o impacto da intervenção, mesmo sendo a via principal na transmissão da leptospirose, conforme descrito na literatura (Khalil et al., 2021; Palma et al., 2022). Em suma, a intervenção não interrompeu todas a vias de transmissão, ao ponto de permitir se observar mudanças reais na exposição a bactéria *Leptospira* no ambiente.

A sazonalidade das chuvas está associada ao risco de leptospirose (Hacker et al., 2020). No entanto, a precipitação pluviométrica ocorrida entre os períodos estudados teve comportamento similar, que proporcionou inúmeros eventos de inundações em locais de infraestrutura de drenagem das águas pluviais precária. Por causa disso, não incluímos na avaliação já que as áreas estudadas são propensas a inundações e a maioria dos participantes relataram inundação do ambiente domiciliar e contato com águas de alagamentos.

Outro fator importante é a duração da persistência de anticorpos anti-*Leptospira* nos residentes. Estudos recentes nas ilhas Fiji, mostraram que o tempo de persistência de anticorpos é 8,33 anos, com taxa individual dos susceptíveis adquirirem infecção ou soroconversão de 3,15%, ao ano (Rees et al., 2022). A heterogeneidade de anticorpos e o menor tempo de acompanhamento sorológico após a intervenção dificultou observarmos reduções significativas na presença de anticorpos. Logo, maior duração de acompanhamento sorológicos, após intervenção, pode ser necessário para medir com precisão a duração da persistência de anticorpos e assim, quantificarmos as taxas reais de decaimento de anticorpos. Uma vez que, não sabemos o momento exato da infecção e talvez seja possível que estejamos avaliando os indivíduos nos seus níveis máximos de títulos de anticorpos.

A maioria dos estudos que avaliam o impacto de intervenções sanitárias estão focados nas doenças diarreias e vem apresentando resultados variáveis quanto a efetividade (Andres et al., 2018; Colford, 2017; International Initiative for Impact Evaluation (3ie), 2020). Por ser este um estudo inédito para a doença leptospirose, não temos como comparar nossos achados e nem propor generalizações para outros ambientes. No entanto, esses resultados são consistentes com achados de outros estudos que avaliaram diferentes intervenções sanitárias para outros desfechos clínicos (Capone et al., 2020; Pickering et al., 2015; Steinbaum et al., 2019; Taylor et al., 2015). Os resultados negativos destes estudos, podem ser associados ao fato que as intervenções em esgotamento e manejo de águas pluviais não serem projetadas para interromper a transmissão de doenças ou prevenir infecções. Mas, são implementadas para acomodar ruas, eliminar resíduos urbanos e evitar inundações (Watson et al., 2022). Por exemplo, obras de canalização e tamponamento dos córregos poluídos pelo lançamento de esgotos domésticos são intervenções inadequados do ponto de vista técnico, sanitário, ambiental e de saúde pública. Uma vez que, essa obra apenas subtrai o córrego poluído das vistas dos residentes das comunidades, sem que represente uma medida de saneamento adequado. Contudo, é preciso projetar estas intervenções em esgotamento na perspectiva da saúde, como meta pública de reduzir doenças e vetores.

As limitações existentes neste estudo dificultaram a compreensão do impacto da intervenção sanitária na saúde humana. A intervenção foi conduzida e gerenciada pelo poder público local, no qual, não foi possível alocar randomicamente as intervenções e definir os pares de controle. Isso dificultou desenvolvermos um desenho de estudo randomizado controlado, que é um estudo padrão-ouro para avaliar impactos de intervenções. Por causa disso, os efeitos das intervenções podem estar sendo mascarados por outros possíveis fatores de confusão atribuída a não aleatoriedade. Este estudo foi conduzido após término das obras de intervenção, todos os participantes avaliados não foram informados quanto ao seu status de intervenção reduzindo os vieses de respostas individuais. Apesar disso, a movimentação dos participantes dentro e fora da coorte poderia trazer um viés em nossas estimativas de efeito caso afetasse as áreas de intervenção ou controle de maneira significativa. No entanto, foi possível observar que o fluxo de indivíduos na coorte não impactou os desfechos avaliados, mantendo as flutuações da positividade estáveis durante todos os seguimentos.

Intervenções em saneamento são urgentes e necessárias em comunidades empobrecidas das grandes metrópoles do mundo. Podemos observar que, adoção de estratégias descentralizadas entres os setores públicos, atuando apenas em um componente do saneamento, não garante o acesso e benefícios duradouro na salubridade ambiental e na saúde humana. A resiliência e sustentabilidade dos programas de intervenções sanitárias requer a integração entre diferentes setores institucionais, como por exemplo gerenciamento dos serviços de esgotamento sanitário e controle de vetores com parte integrante da infraestrutura de saneamento (Cecilia et al., 2023). Outro elemento passível de integração é gestão urbana baseada em bacias hidrográficas, considerando aos aspectos sociais, culturais e políticos do território, visto a ocupação urbana ao longo dos rios e a topografia das comunidades, que prejudicam o escoamento das águas provocando enchentes e alagamentos durantes os períodos de chuvas. Frequentes eventos de inundações e alagamento ampliam a pobreza destas comunidades, já que estas catástrofes naturais geram perdas econômicas e riscos à saúde, expondo a população a diferentes patógenos ambientais, como por exemplo a leptospirose. Por fim, também sugerimos a integração com os setores sociais, ou seja, a participação individual e coletiva da população civil na implementação de obras sanitárias, que é fundamental para a manutenção das instalações sanitárias domiciliares (Nelson et al., 2021; Tsekleves et al., 2022). As ações participativas comunitárias oferecem direito à participação política na tomada de decisão aos usuários do saneamento, garantindo uma prestação dos serviços de água e esgoto mais adequada do que apenas construções de instalações físicas (Beard, 2019). Estas ações combinadas facilitarão a implementação de múltiplas intervenções articuladas, que pode gerar um efeito cascata na dinâmica de transmissão da Leptospirose e outras doenças, uma vez que promove mudanças comportamentais e interrupção das fontes de exposições, reduzindo o risco de doença.

Conclusão

Nosso estudo sugere que obras de canalização e tamponamento de corpos d'agua poluídos por esgotos domésticos não foi eficaz na redução da transmissão de *Leptospira* sp.. Tal fato, certamente, deve-se à própria característica das intervenções que não consideram requisitos técnicos essenciais para o manejo das águas urbanas, especialmente de coleções de águas poluídas por esgotos domésticos. No entanto, nosso estudo indica que a intervenção sanitária realizada na comunidade de Pau da Lima, Salvador, Brasil teve efeito na redução na probabilidade presença de roedores. Concluímos que, intervenções de saneamentos em ambientes carentes e de alto risco de Leptospirose, devem adotar estratégias urgentes e eficazes que interrompam o ciclo de infecção e exposição, limitando o contato dos residentes às múltiplas vias de contaminação. Para isso, deve-se conceber ações integradas de saneamento básico, tecnicamente adequadas e apropriadas às realidades locais, que incluam as componentes do manejo dos resíduos sólidos e limpeza pública, esgotamento sanitário e manejo das águas pluviais e drenagem urbana, além da promoção de ações para higiene domiciliar e peridomiciliar. Tais ações devem ser projetadas e implementadas de forma que garantam a qualidade e durabilidade de suas estruturas, devendo ser mantidas e operadas adequadamente e continuamente. Além disso, faz-se necessário a integração de diferentes setores públicos e sociais como da habitação, educação, trabalho e renda e da atenção primária à saúde e, ainda com a população local e suas organizações. Esse conjunto de ações articuladas e intersetoriais têm o potencial de prevenir e reduzir casos de doenças, como a leptospirose.

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Figuras e Tabelas

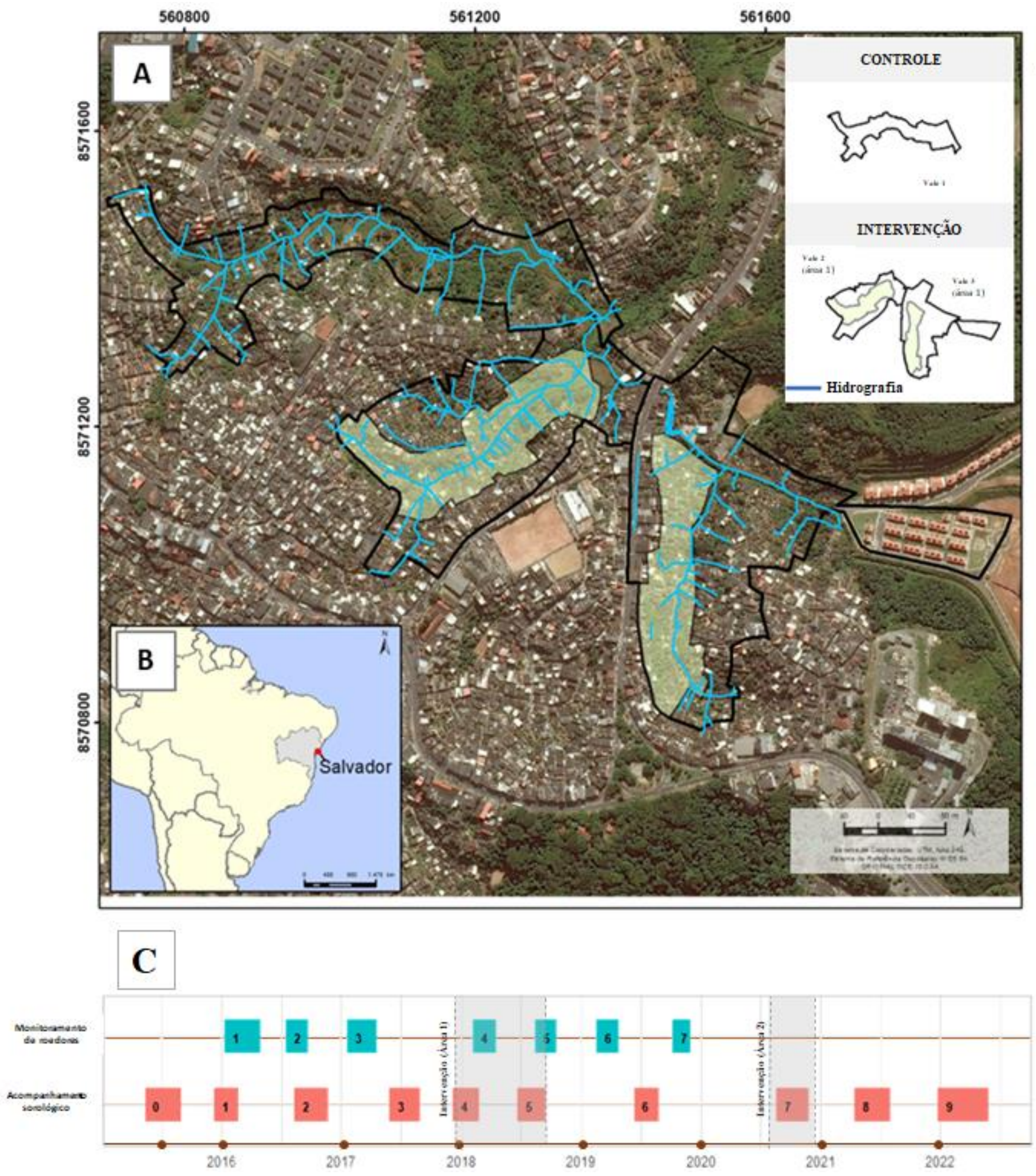

Fig. 1- Área de estudo e linha temporal das amostragens e intervenção. Em **A** (em amarelo), mostramos o polígono da intervenção de canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico no sítio/vale 2 e vale 3 na cidade de Salvador, Bahia (Brasil)

representado na figura **B**. O Vale 1 neste estudo foi considerado área controle. A figura **C**, representa a distribuição dos períodos de coletas de dados de ratos e dados humanos.

Fig. 2- Painel fotográfico da intervenção de canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico no vale 2. As imagens em **A** - Antes da intervenção, **B**- Durante da intervenção e **C** - Depois da intervenção. A intervenções similares ocorreram no vale 3 em períodos diferentes.

Tabela 1- Modelo multivariado de efeitos mistos (binomial) mostrando o efeito da canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico na atividade de roedores. Nível de significância 0,05.

	Infestação de roedores		
Preditores	OR	IC	*p*
Intervenção	**0,4**	**0,33 – 0,49**	**<0,001**
Vale [1]	*Ref.*		
Vale [2]	0,9	0,67 – 1,20	0,464
Vale [3]	**0,65**	**0,49 – 0,86**	**0,003**
Presença de resíduos sólidos	**2,28**	**2,03 – 2,57**	**<0,001**
Presença de vegetação	**1,81**	**1,51 – 2,17**	**<0,001**
Distância ao esgoto (em metros)	**0,99**	**0,99 – 1,00**	**<0,001**

OR: *Odds ratio*; IC-Intervalo de confiança.

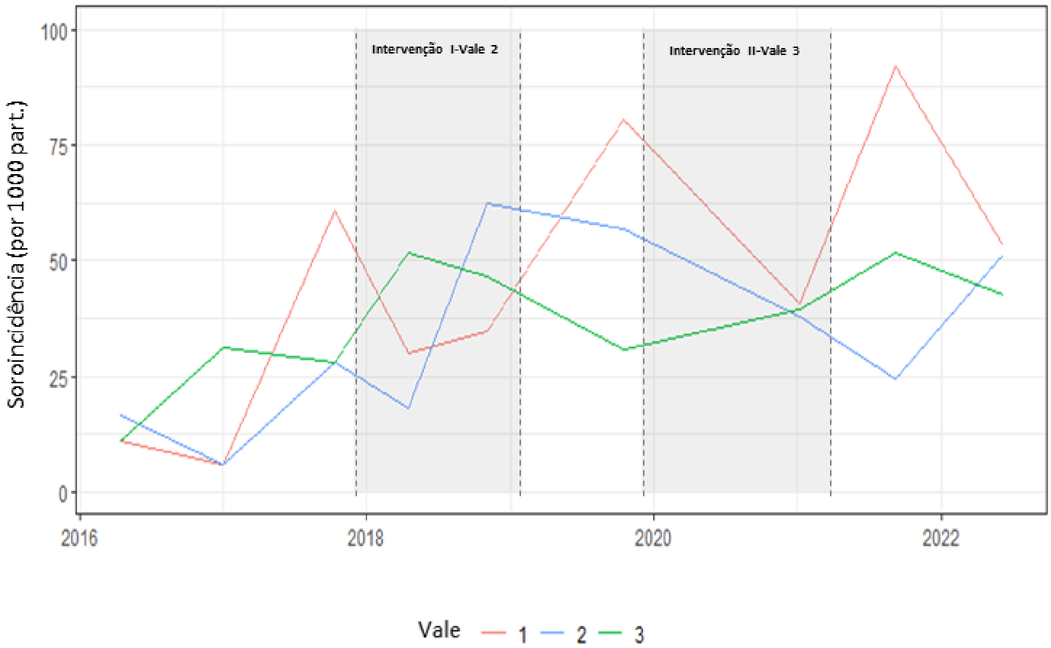

Fig. 3- Soroincidencia de infeccao por *Leptospira*. A infecção foi definida a partir de teste de microaglutinacao, com resultados de título, *cut-off* 1:50. Os indivíduos que obtiveram aumento de 4x no título ou seroconverteram entre os períodos foram considerados positivos. Em **cinza**, mostramos os períodos que ocorreram as intervenções.

Tabela 2- Soroincidencia de infecção por *Leptospira* (por 1000 hab.) entre grupos intervenção (vale 2 e 3) e controle (vale 1) antes e depois da intervenção na comunidade de Pau da Lima, Salvador-Bahia.

Intervenção I-Vale 2							
	Controle (Vale1)			**Intervenção (Vale 2)**			
Período	**Antes**	**Depois**	**%**	**Antes**	**Depois**	**%**	**Risco atribuído (%)**
Período 3 (2017)	60,87	_	_	27,78	_	_	_
Período 6 (2019- 8 meses depois)		80,57	32,36		56,65	71,56	104
Período 7 (2020-2 anos depois)		40,54	-33,39		37,79	36,03	69,43
Período 8 (2021-2,5 anos depois)		91,89	50,96		24,55	-11,63	-62,59
Período 9 (2022-3 anos depois)		53,14	-12,70		51,18	84,23	96,93
Intervenção II-Vale 3							
	Controle (Vale1)			**Intervenção (Vale 2)**			
Período	**Antes**	**Depois**	**%**	**Antes**	**Depois**	**%**	**Risco atribuído (%)**
Período 6 (2019)	80,57	_	_	30,73	_	_	_
Período 8 (2021-4 meses depois)		91,89	14,05		51,91	68,92	54,87
Período 9 (2022-1 ano depois)		53,14	-34,04		42,74	39,08	73,13

Tabela 3- Modelo linear generalizado (binomial) exibindo o efeito da intervenção sanitária na infecção por *Leptospira* no vale 2 e 3, usando o vale 1 como controle. Este modelo considera a soroincidência em cada momento, usando a definição de aumento de 4 vezes no título ou soroconversão (reinfecção). Nível de significância 0,05.

	Intervenção I (Vale 2)			**Intervenção II (Vale 3)**		
Preditores	OR	IC	*p*	OR	IC	*p*
Idade (em anos)	1,03	1,02 – 1,04	**<0,001**	1,02	1,01 – 1,03	**<0,001**
Sexo						
Mulher	Ref.	_	_	Ref.	_	_
Homem	1,51	1,10 – 2,06	**0,0101**	1,76	1,30 – 2,36	**<0,001**
Elevação (em metros)						
0-6,7	Ref.	_	_	Ref.	_	_
6,7-15,6	0,724	0,479 – 1,11	0,129	0,82	0,561 – 1,20	0,304
>15,6	0,494	0,321 – 0,764	**0,0014**	0,683	0,450 – 1,03	0,0688
Vale geográfico						
1	Ref.	_	_	Ref.	_	_
2	0,556	0,290 – 1,08	0,0786			
3				0,809	0,550 – 1,20	0,287
Intervenção						
Antes	Ref.	_	_	Ref.	_	_
Durante	1,08	0,506 – 2,25	0,84	0,878	0,299 – 2,07	0,788
Depois	2,53	1,42 – 4,69	**0,0021**	2,61	1,37 – 4,74	**0,0022**
Durante * vale 2	2,24	0,899 – 5,68	0,0857			
Depois * vale 2	0,911	0,410 – 2,00	0,817			
Durante * vale 3				1,14	0,379 – 3,89	0,82
Depois * vale 3				0,639	0,287 – 1,44	0,274

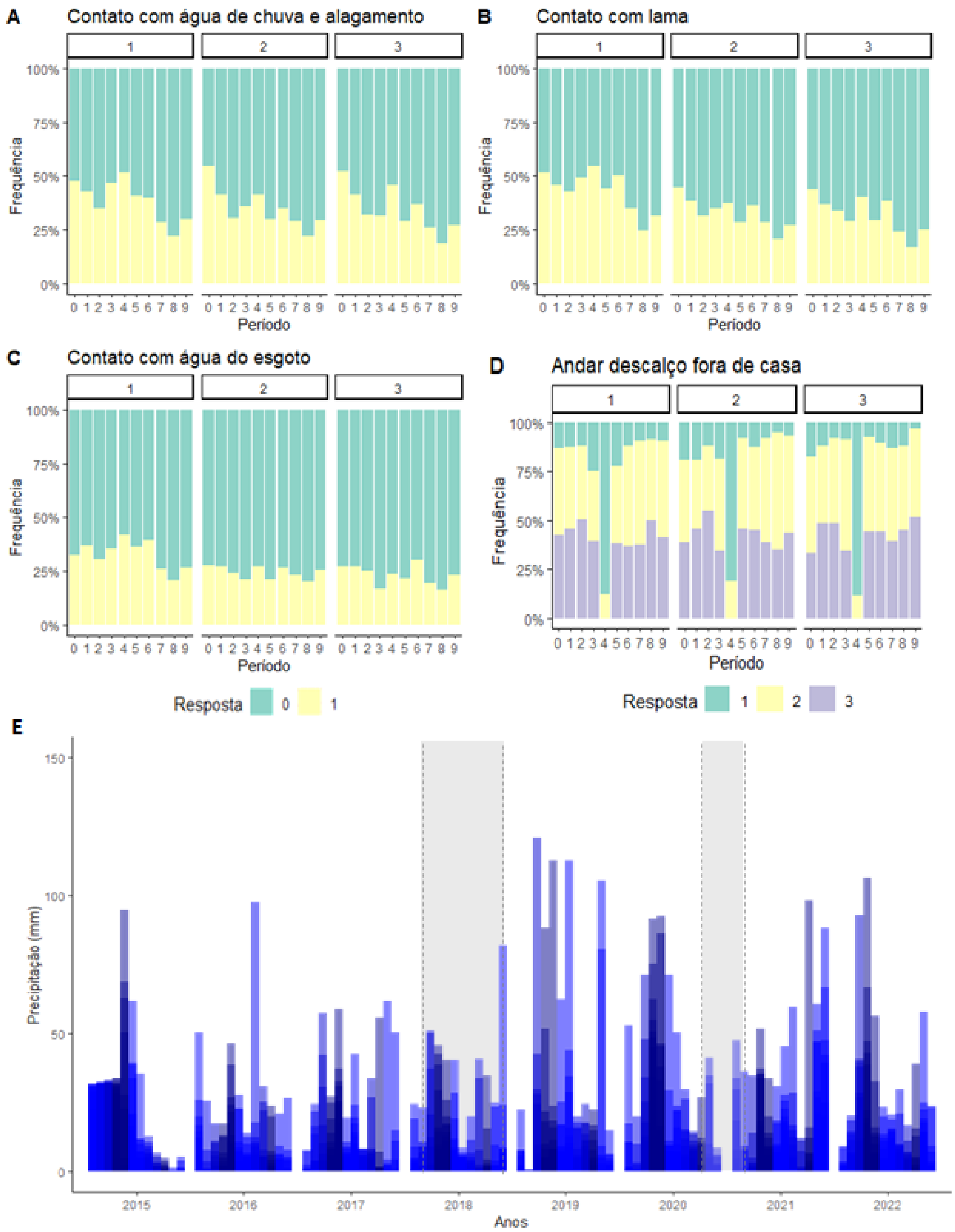

Fig. 4- Frequência das exposições individuais a coleções de águas contaminadas por vale (1,2 e3). Mostramos em **A** proporção de indivíduos que nos últimos seis meses tiveram contato com água da chuva e alagamento; **B** contato lama; **C** contato com água pluviais contaminadas com esgotos domésticos (0-Não e 1-Sim) e em **D** hábito de caminhar descalço fora de casa (1- raramente, 2- as vezes e 3-frequentemente). A precipitação diária em mm durante os períodos de coleta está representada em **E**.

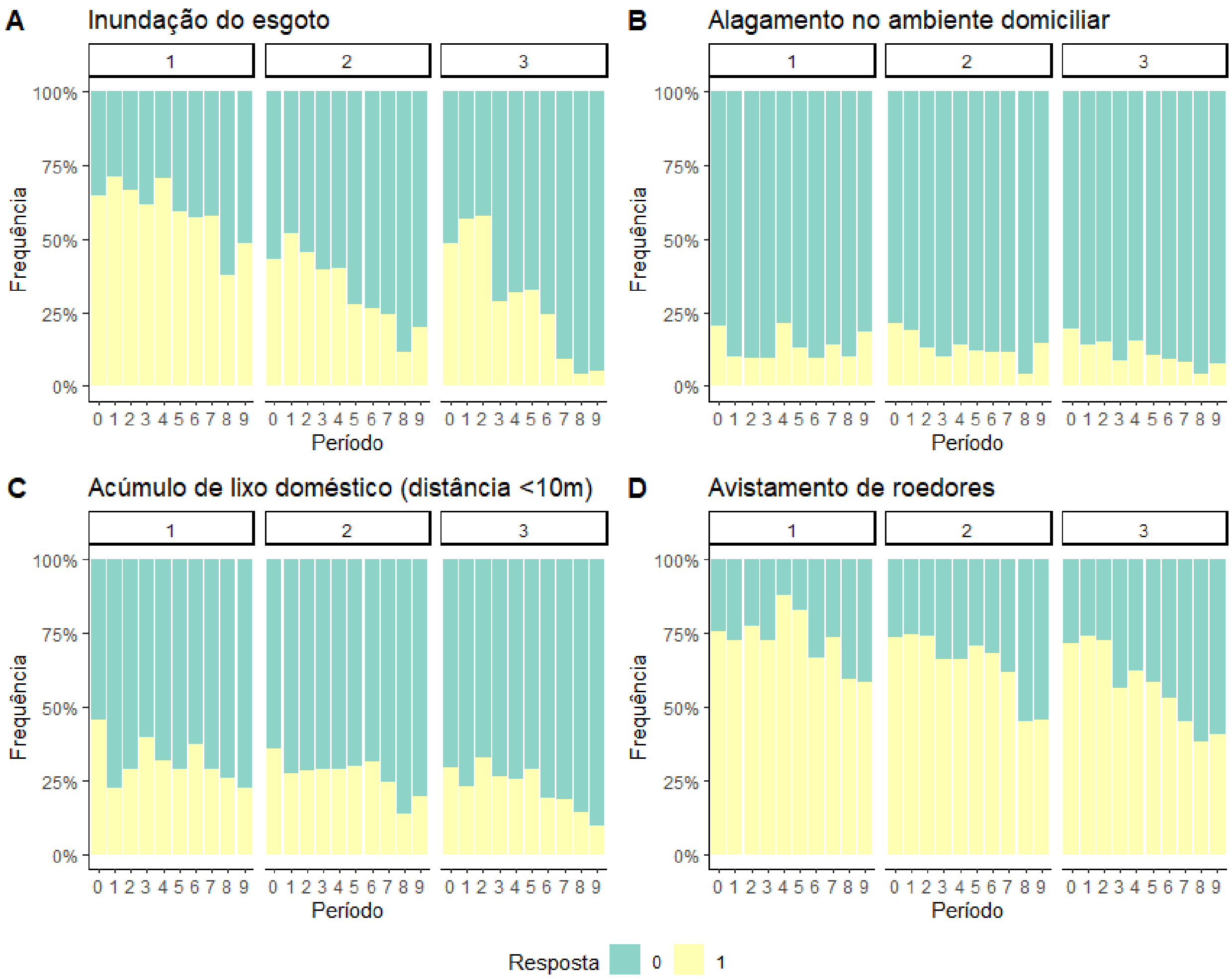

Fig. 5- Frequência das exposições ambientais e avistamento de ratos por vale (1,2 e3). Mostramos em **A** proporção de indivíduos que nos últimos seis meses observaram inundação com águas pluviais com esgotos domésticos (distância 10m em relação ao domicílio) em **A**. No gráfico **B**, mostramos a proporção de indivíduos que afirmaram ter sua casa alagada durante as chuvas; **C** proporção dos indivíduos que vivem em proximidades com acúmulo de lixo e **D** mostramos a proporção de avistamento de ratos no local durante os períodos de coleta.

Material suplementar

Tabela Suplementar 1- Modelo linear generalizado (binomial) exibindo o efeito da intervenção sanitária na infecção por *Leptospira* no vale 2 e 3, usando o vale 1 como controle. Este modelo considera a soroincidência em cada ponto de tempo, usando queda de título ao definir reinfecção. Nível de significância 0,05.

	Intervenção I (Vale 2)			**Intervenção II (Vale 3)**		
Preditores	OR	IC	*p*	OR	IC	*p*
Idade (em anos)	1,03	1,02 – 1,04	**<0,001**	1,02	1,02 – 1,03	**<0,001**
Sexo						
Mulher	Ref.	_	_	Ref.	_	_
Homem	1,67	1,33 – 2,09	**<0,001**	1,88	1,49, 2,37	**<0,001**
Elevação (em metros)						
0-6,7	Ref.	_	_	Ref.	_	_
6,7-15,6	0,545	0,404 – 0,735	**<0,001**	0,749	0,557 – 1,00	0,0541
>15,6	0,481	0,359 – 0,645	**<0,001**	0,598	0,432 – 0,821	**0,0017**
Vale geográfico						
1	Ref.	_	_	Ref.	_	_
2	0,933	0,574 – 1,55	0,78			
3				1,01	0,739 – 1,39	0,953
Intervenção						
Antes	Ref.	_	_	Ref.	_	_
Durante	0,853	0,472 – 1,53	0,59	0,675	0,275 – 1,42	0,341
Depois	1,41	0,853 – 2,36	0,19	1,63	0,898 – 2,82	0,0915
Durante * vale 2	1,41	0,704 – 2,84	0,33			
Depois * vale 2	1,23	0,667 – 2,26	0,5			
Durante * vale 3				1,62	0,678 – 4,33	0,301
Depois * vale 3				0,671	0,333 – 1,38	0,268

Fig. Sup. 1- Painel fotográfico da intervenção de canalização e tamponamento dos corpos d`água poluídos com esgoto doméstico no vale 3. As imagens em **A** - Antes da intervenção, **B**- Depois da intervenção.

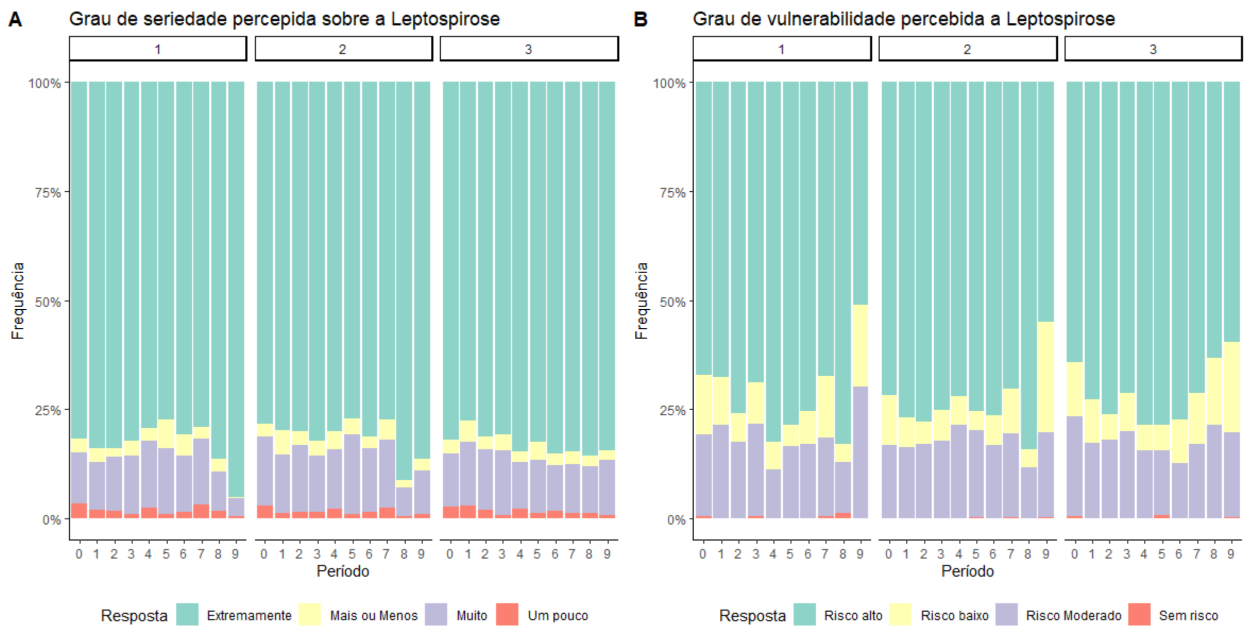

Fig. Sup. 2- Percepção de risco dos participantes entre vales (1,2 e3). A seriedade da doença Leptospirose (A) e vulnerabilidade (B) percebidas dos participantes foi observada pelo score de pontuação obtidos para cada indivíduos. **Nota:** O grau de vulnerabilidade da doença foi agrupado em percentis de 20% (1 pontos) -risco baixo, 40-60% (2-3 pontos) - moderado e acima de 80% (4-5 pontos) - risco alto e zero pontos foram atribuídos aos indivíduos que afirmaram sem risco. Os escores de seriedade da doença, foram classificadas de acordo com pontuações de 0-10 pontos, sendo 0- nem um pouco; 1-4- pouco; 5- mais ou menos; 5-7- muito seria e 7-10- extremamente seria.

Fig. Sup. 2- Gráfico da variação da positividade de infecção por *Leptospira* entre períodos e vales.

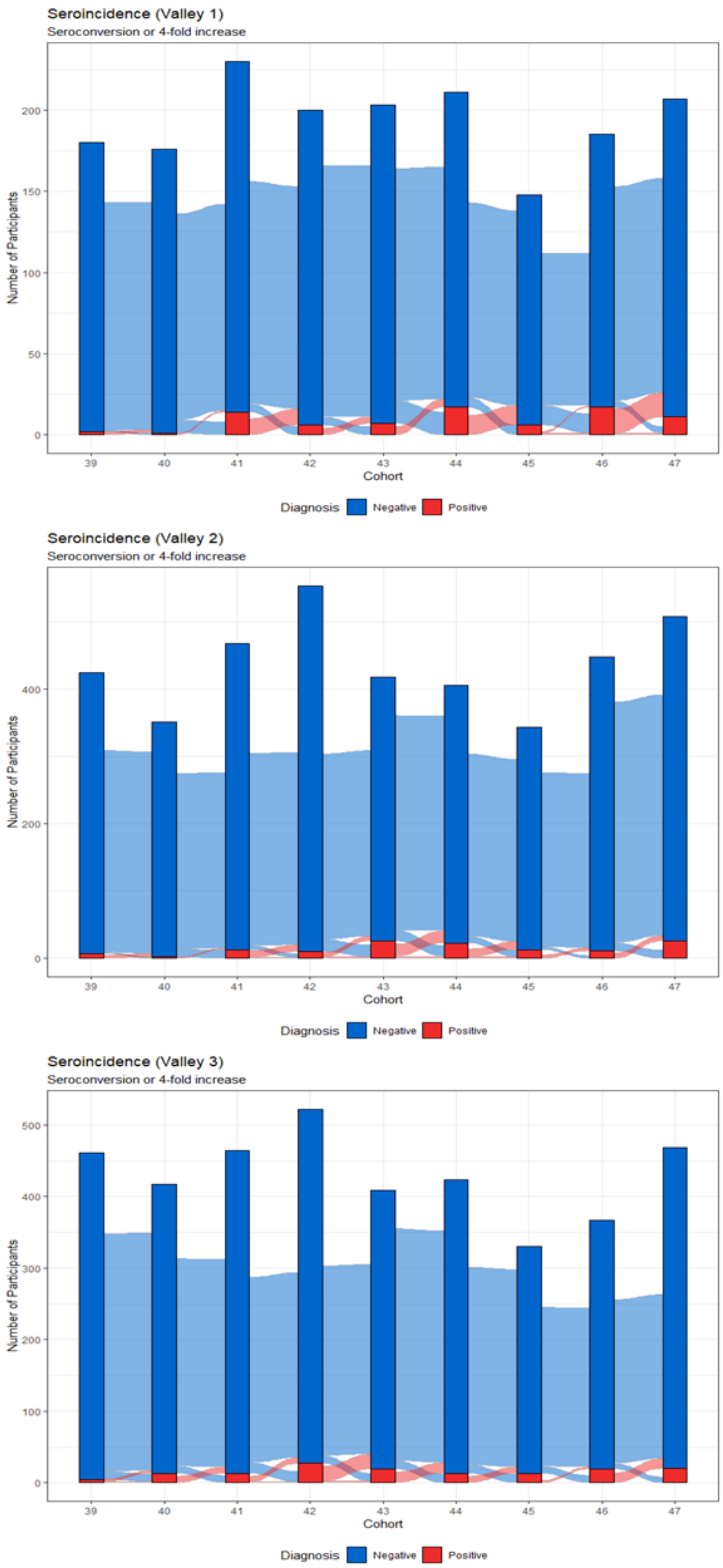

Fig. Sup. 2- Gráfico da variação da positividade de infecção por *Leptospira* entre períodos e vales.

Capítulo II

Artigo publicado no periódico *Environmental Science & Technology*

Fator de impacto: 11.357 (2021); Qualis A1 (Biodiversidade)

DOI: 10.1021/acs.est.1c04916

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

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 poor communities world-wide. In urban slums, leptospirosis is associated with deficient sanitary infrastructure. Yet, the role of sewerage in the reduction of the environmental contamination with pathogenic *Leptospira* has not 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 soils surrounding conventionally-closed sewers (governmental interventions) were 3 times less likely to contain pathogenic *Leptospira* (inverse OR 3.44, 95% CI: 1.66-8.33; $p<0.001$) and contained a 6 times lower load of the pathogen (0.82 $\log_{10}$ units difference, $p<0.01$) when compared to their open counterparts. However, no differences were observed in community-closed sewers (poor quality closings performed by the slum dwellers). Human fecal markers (BacHum) were positively associated with pathogenic *Leptospira* even in closed sewers and 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, deficient 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.

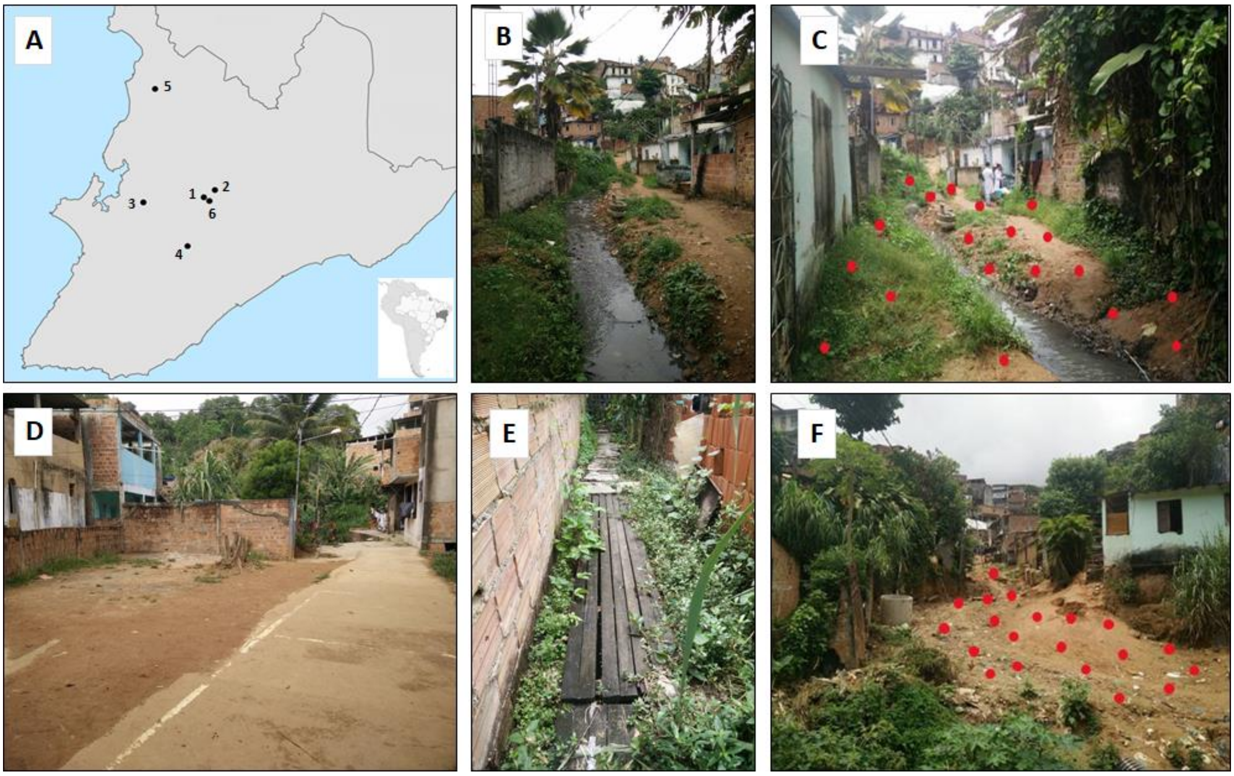
Sanitary interventions to close open sewers are an alternative to reduce exposures to environmental sources of *Leptospira* [16,24], given the lack of efficacious vaccines for human use [1,25] and the limited success of rodent control strategies due to regrowth after extermination [26,27]. Sewerage construction 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 sewerage in the environmental contamination with pathogenic *Leptospira* in urban slums. 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 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 deficient 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). The selected sites were similar regarding the presence of impervious surfaces, exposed soil and mud and the presence of vegetation and trash. 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. In all sites the open section was located downstream of the closed section.

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, closed and open contiguous 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 separated by approximately 20m 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.

All samples were collected in the first of December of 2018, during the typically less rainy season in Salvador (Brazilian Institute of Meteorology). During the collection period, no big rainfall events (>20mm/day) were recorded in the meteorological stations close to the selected urban slums. All collections were performed a full day after the last recorded rainfall.

Samples in open and closed sections at each site were collected the same day between 10 am and 12pm.

Each collection point, an area of ~400 cm^2 was cleared from surface rocks and vegetation debris, and ~25g of subsurface soil were collected at a depth of approximately 5 cm, stored in aseptic containers, transported to the laboratory and processed within 4h of collection as described previously with minor modifications.[32] Soil samples were mixed with a sterile spatula for 5min to homogenize and a subsample of 5g was transferred to a 50 mL sterile polypropylene tube. Then, 40 mL of sterile double-distilled water was added to each 5g sample and shaken with a horizontal vortex adaptor at maximum speed for 2 min. Samples were centrifuged at 100 rcf for 5 min, the supernatant (approximately 45 mL) 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 in sterile 50mL polypropylene tubes at the end of the closed and open sections in each site. Sewage samples were homogenized by inversion and a 40-mL aliquot was centrifuged at 15,000 rcf for 20 min at 4 ˚C. The supernatant was discarded and the pellet was recovered, and frozen at -20 ˚C.[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] This qPCR assay has been shown to specifically detect species from the subclade P1 in complex environmental samples. [16,32] To determine the levels of human fecal contamination, we used the BacHum TaqMan qPCR assay.[33,34] Calibration curves based on genomic DNA from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 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.[35,36] 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).[35] 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.[37]

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 wet 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 used Fischer's exact test to compare rat activity between open and closed sewer sections and the environmental variables between sites.

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). [38] We also included other covariates such as distance to the sewer, soil moisture, rat activity (presence/absence of rat marks and number of rat marks) and human fecal markers (BacHum), and a randomization factor for the sampling site. [39,40] 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 $\Delta AIC < 2$ was selected as the best model using the *dredge ()* function of the R MuMIn package. [41,42] 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 [43], 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%, 95% CI =24.4−39.9%) than in their closed counterparts (18.4%, 95% CI =12.7%−26.8%) (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 with 54.2% (13/24), whereas the lowest was found in the closed section of Site 2 with 0.0% (0/24) (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).

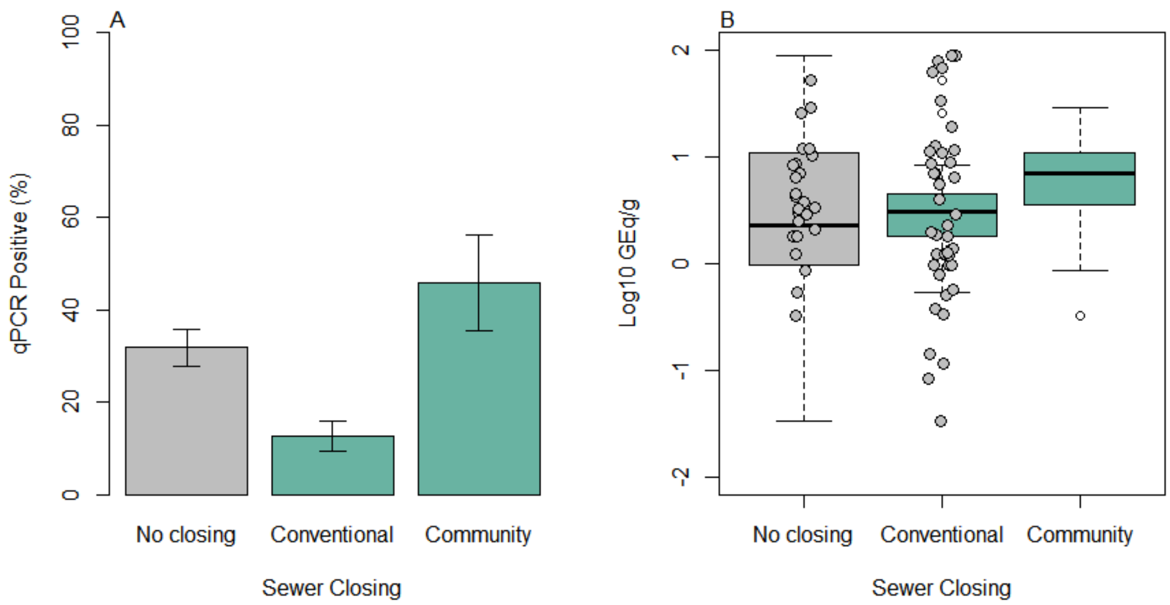
Table 1. Occurrence and concentration of pathogenic *Leptospira* in soils surrounding open anclosed sewers in the six Brazilian urban slums. Closed sewers are classified based on the type of closing: conventional or community-based.

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 (median and interquartile ranges). 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 (at the end of the closed and the open sections), at least one of the two paired samples collected was positive, indicating that sewage is a potential source of pathogenic *Leptospira* to soils surrounding sewers.

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 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 when compared to their open counterparts. However, no significant differences were observed when comparing open and 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 are implemented by the local government and 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 [16,24,44], its canalization may eliminate spillage contaminations in soil. However, the imperfect closure of community-based interventions that consist of suboptimal closings made with poor-quality construction materials such as wood boards or concrete panels might 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*) [45]. Interestingly, we observed a correlation between BacHum and pathogenic *Leptospira* in open, but also closed sewer sections. This indicates that besides the adjacent sewers, 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 [32,46,47], 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.

The concentrations of pathogenic *Leptospira* in the collected soils were generally low in the six urban areas studied (mean $\log_{10}$ 0.56±0.8 and 2.00-1.62×10^3 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. [16,24,32,48]. 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 [8,49] 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 [50–52]. 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 [5,53,54] and rat presence is commonly reported as a factor for leptospirosis infection. [37,55] 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 [56,57] 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. Other important factors that may affect the presence, survival and distribution of the pathogen in soil such as rainfall, storm water hydrology, and elevation will need to be considered in future studies. 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), Fundação de Amparo á Pesquisa do Estado da Bahia (FAPESB/JCB0020/2016), 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. Fábio Neves Souza participated in this study under a FAPESB doctorate scholarship.

Supporting Information: Supplementary methods for qPCR and inhibition testinc, supplementary tables and figures for the presence and concentration of *Leptospira*; and supplementary tables for the univariate and multivariate models.

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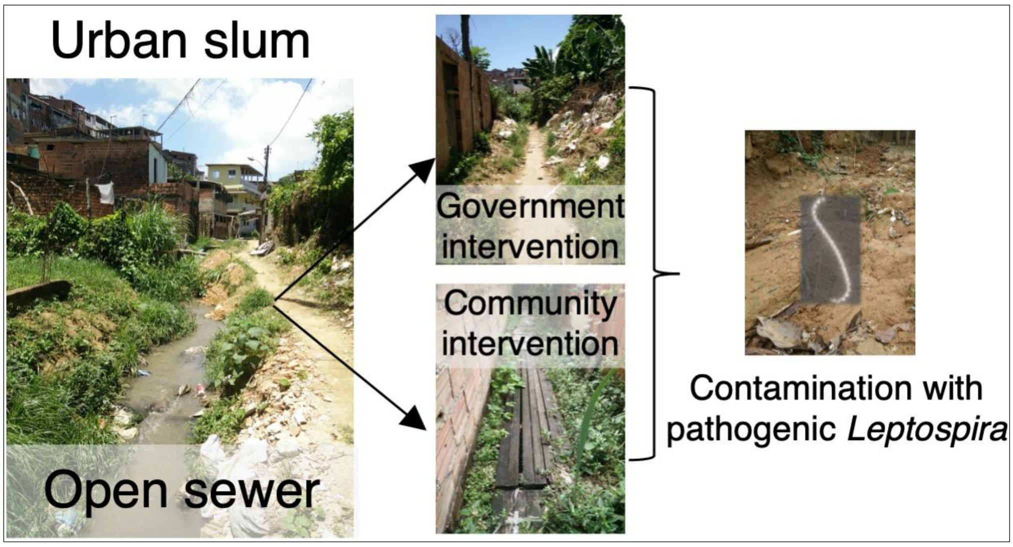


Supporting Information for:

1. METHODS

qPCR

Pathogenic *Leptospira* was quantified using a TaqMan® assay targeting the *lipL32* gene with minor modifications (Stoddard et al., 2009). BacHum was quantified as described by Keldare et al., 2007 with minor modifications. Reaction mixtures (25 µL) contained 12.5 µL Platinum® qPCR SuperMix (Life Technologies), 0.2 µg/µL of bovine serum albumin (Ambion) and 5 µL of DNA template. For *lipl32* reactions contained 500 nM of primers LipL32-45F and LipL32-286R (Table S3) and 100 nM of LipL32-189P probe. For BacHum, reactions contained 400nM of primers BacHum-160f and BacHum-241r and 80nM of probe BacHum-193p. Amplifications were performed in a 7500 Fast Real-Time PCR System (Applied Biosystems). The thermal cycling conditions consisted of an initial setup of 2 min at 50°C, followed by 2 min at 95°C, and 40 cycles of amplification (15 s at 95°C and 1 min at 60°C). All samples, controls and calibrators were run in duplicate. Non-template controls were randomly included in each row of the 96-well plates to discard the presence of contaminating DNA. All negative controls (extraction blanks and non-template controls) were negative in all cases

Calibration curves

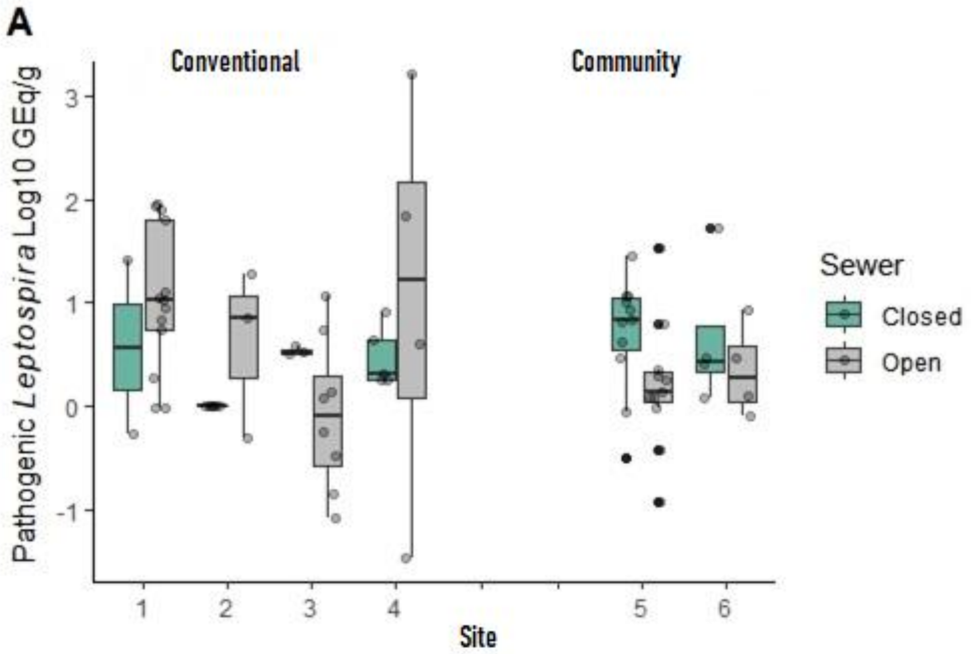
Genomic DNA from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 was extracted using the automated Maxwell® 16 Cell DNA Purification Kit (Promega). For BacHum, a circular plasmid containing the BacHum target was transformed in *E. coli* and purified with the Qiagen MiniPrep kit. *Leptospira* gDNA and BacHum plasmid were quantified with Nanodrop 2000 (Thermo Fisher Scientific). Each DNA was serially 10-fold diluted to construct calibration curves with concentrations ranging from 2×10^9 to 2×10^2 GEq/mL. Genomic equivalents for *Leptospira* were calculated based on the genome size of the Fiocruz L1-130 strain (Nascimento et al., 2004). Calibration curves were included in each qPCR run and efficiencies were always higher than 92.5% for *lipl32* or 94.5% for BacHum.

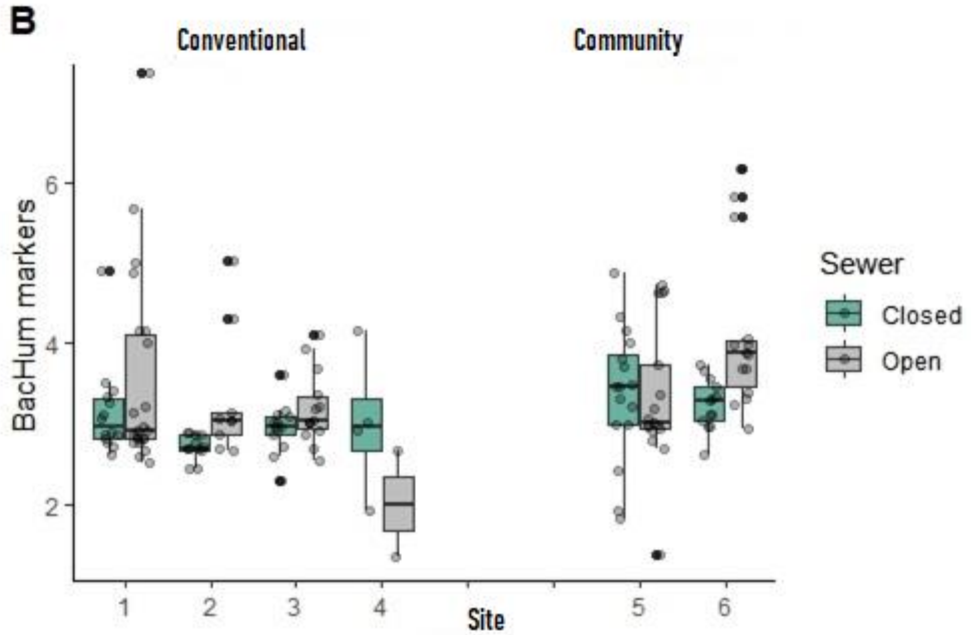
Inhibition testing

A plasmid DNA construct developed previously (Casanovas-Massana et al., 2018) was used to monitor for inhibition in *lipl32* reactions. qPCR inhibition was monitored in each environmental sample by qPCR using the designed IAC plasmid. Reaction mixtures (25 µL) contained 12.5 µL Platinum® qPCR SuperMix (Life Technologies), 500 nM of each primer LipL32-45F and LipL32-286R, 100 nM of LipL32-189IAC probe, 0.2 µg/µL of bovine serum albumin (Ambion), 5 µL of DNA template and 200 copies of the IAC plasmid. Amplifications were performed in a 7500 Fast Real-Time PCR System (Applied Biosystems). The thermal cycling conditions consisted of an initial setup of 2 min at 50°C, followed by 2 min at 95°C, and 40 cycles of amplification (15 s at 95°C and 1 min at 60°C). As a non-inhibition control, each plate included 5 wells with 200 copies of the IAC plasmid but no DNA template. Non-IAC controls were randomly included in each row of the 96-well plates to discard the presence of contaminating DNA. As criteria for inhibition, we established a limit of 2 standard

deviations above the mean quantification cycle (C_q of 32.63 + 1.51), which was based on C_q values obtained from 30 control amplifications of IAC in ultrapure water in 7 independent qPCR runs. DNA extracts were assumed to be free of qPCR inhibitors when their mean C_q value fell below that limit. The mean IAC Ct for 3 samples fell over that limit. Such DNA extracts were diluted 1:2 and re-tested for both qPCR systems (lipL32 and IAC).
For BacHum, samples were tested in serial ten-fold dilutions (10^{-1}, 10^{-2} and 10^{-3}). In all cases, dilution 10^{-2} resulted in a higher target concentration estimation, and therefore was used for further calculations.

Supplemental Fig 1. Concentration of (A) pathogenic *Leptospira* and (B) BacHum markers in soils surrounding open and closed sewers from the six slum communities studied.





Supplemental Table 1- Occurrence and concentration of BacHum markers 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 sewer intervention	BacHum positivity rate (n and %)[a]		BacHum markers concentration (Mean Log_{10} and SD)	
	Open sewer	**Closed sewer**	**Open sewer**	**Closed sewer**
Conventional				
Site 1 – Pau da Lima 1	22 (91.7%)	14 (58.3%)	3.53 (1.23)	3.15 (0.58)
Site 2 – Sete de Abril	9 (37.5%)	12 (50.0%)	3.31 (0.80)	2.71 (0.16)
Site 3 – Campinas de Pirajá	14 (58.3%)	13 (54.2%)	3.18 (0.45)	2.93 (0.31)
Site 4 – Tancredo Neves	2 (8.3%)	4 (17.39%)	2.00 (0.94)	3.00 (0.92)
Total governmental	47 (49.0%)	43 (45.2%)	3.32 (0.99)	2.95 (0.47)
Community-based				
Site 5 – Nova Constituinte	17 (70.8%)	16 (66.7%)	3.33 (0.89)	3.37 (0.83)
Site 6* – Pau da Lima 2	14 (93.3%)	13 (81.2%)	4.11 (1.00)	3.24 (0.31)
Total community	31 (79.5%)	29 (72.5%)	3.68 (1.01)	3.31 (0.64)
Overall	78 (57.8%)	72 (53.3%)	3.46 (1.01)	3.09 (0.58)

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

*16 samples were collected in each area from site 6.

Supplemental Table 2- Models univariate/bivariate and multivariate of the probability of pathogenic *Leptospira* based on lipl32 qPCR in soil samples using linear mixed model (glmm, family=binomial) associated with pathogenic Leptospira positivity to environmental and rat variables.

	Model of pathogenic Leptospira present					
	Univariate Analysis			Multivariate Analysis		
Predictors	*OR*	*CI*	*p*	*OR*	*CI*	*p*
Type of sewer						
(Intercept)	0.20	0.10 – 0.40	***	0.38	0.18 – 1.35	*
Closed (Ref.)	–	–	–			
Open	**2.17**	**1.21 – 3.89**	**			
Type of closing						
(Intercept)	0.22	0.22 – 0.79	**			
No closing (Ref.)	–	–	–			
Conventional intervention	**0.26**	**0.11 – 0.54**	***	**0.27**	**0.11 – 0.82**	**
Community intervention	1.35	0.46 – 3.98		1.12	0.38– 2.65	
Distance[(a)] n=175						
(Intercept)	0.36	0.18 – 0.73	**			
Distance(m)	1.05	0.95 – 1.16				
(Intercept)	0.36	0.18 – 0.72	**			
Distance:Closed Sewer	1.07	0.89 – 1.28				
Distance:Open Sewer	1.05	0.95 – 1.16	#			
Proximity to other open sewers[(b)] n=239						
(Intercept)	0.30	0.15 – 0.63	**			
Presence of sewage nearby	1.00	0.99 – 1.01				
(Intercept)	0.21	0.08 – 0.52	***			
Presence of sewage nearby:Closed sewer	1.00	0.99 – 1.01				
Presence of sewage nearby:Open sewer	1.09	1.01 – 1.18	**			
Fecal human markers[(c)]						
(Intercept)	0.28	0.17 – 0.48	***			
Concentration fecal human markers	**1.16**	**1.06 – 1.28**	***	**1.15**	**1.05 – 1.26**	**
(Intercept)	0.28	0.17 – 0.47	***			
Concentration fecal human markers:Closed sewer	1.12	0.99 – 1.26				
Concentration fecal human markers:Open sewer	1.21	1.07 – 1.37	**			
Rodents (markers)[(d)]						
Intercept)	0.74	0.26 – 2.11				
Rats activity	**0.96**	**0.93 – 0.99**	**	1.00	0.94– 1.03	
(Intercept)	0.76	0.27 – 2.13				

Rats activity : Closed sewer	0.95	0.92 – 0.98	**
Rats activity : Open sewer	0.97	0.94 – 1.00	#
Moisture(%) n=206			
(Intercept)	0.22	0.08 – 0.59	**
Moisture	1.02	0.98 – 1.07	
(Intercept)	0.16	0.05 – 0.56	**
Moisture:Closed sewer	1.00	0.95 – 1.06	
Moisture:Open sewer	1.08	1.02 – 1.15	**

OR = Odds Ratios; CI = Confidence intervals
p-values' = significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘#’ 0.1 ‘ ’ 1

Supplemental Table 3- Model univariate of concentration of pathogenic Leptospira based on lipl32 qPCR (in Log(GC/g or ml)) in soil samples using linear mixed model (glmm with family=Gaussian (link='identity') associated with pathogenic Leptospira positivity to environmental and rat variables.

	Model of concentration of pathogenic Leptospira (Log)					
	Univariate Analysis			Multivariate Analysis		
Predictors	*Estimates(β)*	*CI*	*p*	*Estimates(β)*	*CI*	*p*
Type of sewer						
(Intercept)	-3.14	-3.69 – -2.60	***	-2.42	-3.20 – -1.59	***
Closed (Ref.)						
Open	**0.59**	**0.12 – 1.05**	**			
Type of closing						
(Intercept)	-2.57	-3.05 – -2.110	***			
No closing (Ref.)						
Conventional intervention	**-0.91**	**-1.44 – -0.39**	***	**-0.67**	**-1.41 – -0.13**	**
Community intervention	0.21	-0.54 – 0.98		0.093	-0.67 – 0.94	
Distance[(a)]						
(Intercept)	-2.63	-3.37 – -1.88	***			
Distance(m)	0.02	-0.08 – 0.13				
(Intercept)	-2.64	-3.38 – -1.90	**			
Distance:Closed Sewer	0.06	-0.13 – 0.25				
Distance:Open Sewer	0.02	-0.08 – 0.12				
Proximity to other open sewers[(b)] n=239						
(Intercept)	2.82	-3.44 – -2.21	***			
Presence of sewage nearby	0.00	-0.01 – 0.01				
(Intercept)	-3.00	-3.69 – -2.32				
Presence of sewage nearby:Closed sewer	0.00	-0.01 – 0.01				
Presence of sewage nearby:Open sewer	0.05	-0.01 – 0.11				
Fecal human markers[(c)]						
(Intercept)	-2.87	-3.29 – -2.45	***			
Concentration fecal human markers	**0.13**	**0.06 – 0.19**	***	**0.11**	**0.046– 0.18**	***
(Intercept)	-2.88	-3.30 – -2.46	***			
Concentration fecal human markers:Closed sewer	0.09	-0.00 – 0.18	#			
Concentration fecal human markers:Open sewer	0.16	0.07 – 0.25	**			
Rodents (markers)[(d)]						
Intercept)	-2.01	-2.90 – -1.13	***			
Rats activity	**-0.04**	**-0.06 – -0.01**	**	-0.01	-0.04 – 0.02	
(Intercept)	-2.03	-2.88 – -1.18	***			

Rats activity :Closed sewer	-0.04	-0.06 – -0.02	***
Rats activity :Open sewer	-0.03	-0.05 – -0.00	**
Moisture(%)			
(Intercept)	-3.11	-3.97 – -2.26	***
Moisture	0.02	-0.02 – 0.06	
(Intercept)	-3.18	-4.12 – -2.25	***
Moisture:Closed sewer	0.00	-0.04 – 0.04	
Moisture:Open sewer	0.05	0.01 – 0.10	**

β = beta estimate; CI = Confidence intervals

p-values' = significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '#' 0.1 ' ' 1

Supplemental Table 4: Summary of model performances in explaining the probability and concentration pathogenic *Leptospira*. All the plausible models are shown in bold.

Models Selection	*AICc*	*ΔAICc*	*wi*
Pathogenic Leptospira present			
i. y ~ Type of closing+Concentration fecal human	**284.00**	**0.00**	**0.61**
ii. y ~ Type of closing+Concentration fecal human +Rats activity	286.00	2.08	0.21
iii. y ~Concentration fecal human +Rats activity	287.00	3.13	0.12
iv. y ~ Concentration fecal human	290.00	6.43	0.02
v. y ~ Type of closing	292.00	8.15	0.01
vi. y ~ Type of closing+Rats activity	293.00	9.21	0.00
vii. y ~ Rats activity	294.00	10.10	0.00
viii. y ~ 1	300.00	15.76	0.00
Concentration of pathogenic Leptospira			
i. y ~ Type of closing+Concentration fecal human	**1133**	**0.00**	**0.82**
ii. y ~ Type of closing	1137	4.58	0.08
iii. y ~Concentration fecal human	1138	5.58	0.05
iv. y ~ Concentration fecal human+Rats activity	1140	7.53	0.01
v. y ~ Type of closing+Concentration fecal human+Rats activity	1141	8.87	0.00
vi. y ~ Rats activity	1144	11.13	0.00
vii. y ~ 1	1144	11.30	0.00
viii. y ~ Type of closing+Rats activity	1145	12.19	0.00

Capítulo III

Artigo submetido ao periódico *BMJ Open*

Fator de impacto: 2.496 (2021); Qualis A1 (Saúde Coletiva e Ciências Biológicas III)

Simplified sewerage to prevent urban leptospirosis transmission: a cluster non-randomized controlled trial protocol in disadvantaged urban communities of Salvador, Brazil

Simplified sewerage to prevent urban leptospirosis transmission: a cluster non-randomized controlled trial protocol in disadvantaged urban communities of Salvador, Brazil

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ABSTRACT

Introduction

Leptospirosis is a globally distributed zoonotic and environmentally mediated disease that has emerged as a major health problem in urban slums in developing countries. Its etiological agent is bacteria of the genus *Leptospira*, which are mainly spread in the urine of infected rodents, especially in an environment where adequate sanitation facilities are lacking, and it is known that open sewers are key transmission sources of the disease. Therefore, we aim to evaluate the effectiveness of a simplified sewerage intervention in reducing the risk of exposure to contaminated environments and *Leptospira* infection and to characterize the transmission mechanisms involved.

Methods and Analysis

This matched quasi-experimental study-design using non-randomized intervention and control clusters was designed to assess the effectiveness of an urban simplified sewerage intervention in the low-income communities of Salvador, Brazil. The intervention consists of household-

level piped sewerage connections and community engagement and public involvement activities. A cohort of 1,400 adult participants will be recruited and grouped into eight clusters consisting of four matched intervention-control pairs with approximately 175 individuals in each cluster in baseline. The primary outcome is the seroincidence of *Leptospira* infection assessed through five serological measurements: one pre-intervention (baseline) and four post-intervention. As a secondary outcome, we will assess *Leptospira* load in soil, before and after the intervention. We will also assess *Leptospira* exposures before and after the intervention, through transmission modeling, accounting for residents' movement, contact with flooding, contaminated soil and water, and rat infestation, to examine whether and how routes of exposure for *Leptospira* change following the introduction of sanitation.

Ethics and dissemination

This study protocol has been reviewed and approved by the ethics boards at the Federal University of Bahia and the Brazilian National Research Ethics Committee. Results will be disseminated through peer-reviewed publications and presentations to implementers, researchers, and participating communities.

Trial registration number: Brazilian Clinical Trials Registry, RCT trial RBR-8cjjpgm.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The first rigorous controlled intervention study for the effect of sanitation on Leptospira transmission the findings intend to guide future sanitation policies in communities with similar socio-economic status and environmental contexts.
- This is a cluster-based non-randomized controlled trial with intervention clusters receiving a simplified sanitation intervention and matched control clusters no intervention.

- The alliance between the natural and social sciences and the potential that it has to deepen community perceptions about leptospirosis and interventions before and after, as well as involvement in it in some decision-making processes in the improvement of their health, wellbeing, and sanitation.
- The allocation is non-randomized with selection based on intervention criteria determined by the implementer may have diluted intervention effects.
- Intervention will be carried out by the local public water and sanitation company, following their own planning, with no input or interference from the research team in the schedule or implementation.

INTRODUCTION

Globally, leptospirosis is a leading zoonotic disease in terms of morbidity and mortality [1], causing >1 million cases and >50,000 deaths yearly [1–3]. This zoonosis is an environmental and sanitation-related disease [4] characterized by annual epidemics during periods of increased rainfall and flooding [5,6]. Infection occurs frequently following exposure to water and soil that has been contaminated with the urine of infected animals [7,8]. Leptospirosis is an emerging disease in urban areas of most low- and middle-income countries (LMICs) [1], where a significant fraction of the population lives in disadvantaged urban communities (often called slums or *favelas* in Brazil) [9]. In those settings, poverty, precarious housing conditions, overpopulation, and lack of basic sanitation enable a high density of rats (the main urban reservoir hosts [10–13]) and high leptospirosis transmission [14,15]. As the population living in urban disadvantaged communities will double to 2 billion by 2025 [16] the burden of leptospirosis is expected to increase.

Public health intervention strategies to prevent leptospirosis transmission are essential and must target the infrastructure deficiencies to prevent or diminish human exposure to environmental contamination sources. Governmental interventions focused on improving sanitation are critical for reducing the burden of leptospirosis as well as other diseases, like diarrheal [4,17]. However, these interventions, especially in disadvantaged urban communities, are not a priority or foreseeable soon considering the high cost of providing and maintaining such sanitation infrastructure. Exclusion of the disadvantaged urban communities' health issues in the government's political agenda [18–20], contributes to historical and structural disparities. Brazil, since the eighties, has been recognized as a global leader in the development of cost-effective water and sanitation systems for densely populated urban settings [21,22]. These systems, called simplified or “condominial” water and sewerage systems, are designed to be installed in densely populated, informal communities that are often situated in the kind of hilly terrain, common in Brazilian *favela* communities. The simplified sewerage approach is unique because it redefines the service unit as a community block rather than an individual household. It uses smaller pipes and inspection chambers (or cleanouts) at key junctions to facilitate installation and maintenance [21,22]. The system design reduces costs by decreasing the length of the trenches needed for sewerage lines and by using smaller pipes made from less expensive materials such as PVC (polyvinyl chloride). The installation of this alternative system can cost 50-70% less than a conventional system [21]. The lower costs associated with the installation of simplified systems makes these systems a more feasible alternative to conventional sewer systems in poor, resource-constrained settings. However, the simplified sewerage approach requires intensive community mobilization and engagement [23] as it is residents who take on leadership roles and have responsibility for decision-making.

Health impact evaluations of simplified sewerage approach in preventing disease has previously been limited to diarrheal diseases [24]. The evaluation of such interventions is challenging because of methodological problems such as cost and time of implementation, the requirement of large sample sizes, difficulties with randomization of the intervention allocation, and confounding factors (e.g., rodent infestation and Social-Economic Status). Contact with environments contaminated with sewer overflow or residents' exposure to open sewerage near the household can influence the degree of individual exposure to *Leptospira* bacteria[14]. Humans exposure to *Leptospira* contamination through poor sanitation is mediated by individual behavior, socio-economic and environmental factors, rainfall, and flooding [4]. Therefore, the mechanisms of *Leptospira* transmission have different but important components of the environmental transmission route, for which their relative contribution is unknown because of confounding factors. Only studies that integrate a rigorous characterization of contamination sources (*Leptospira* load) and residents' movement could separate their effects and understand how source reduction interventions impact those transmission mechanisms. Determining the contribution of each of these mechanisms to *Leptospira* infection would help us to simultaneously understand the relationships and pathways that drive transmission [4]. This information is key to making interventions generalizable to other environmentally transmitted diseases that affect urban disadvantaged populations.

In this protocol, we describe an evaluation of a simplified sewerage intervention and community engagement with a quasi-experimental study-design using non-randomized intervention and control clusters. We aim to assess the longitudinal impact of a simplified sewerage intervention on the incidence and transmission mechanisms of *Leptospira* infection. In addition, we intend to investigate the impact of these interventions on reducing other water and environmental-related transmissible diseases.

Research aims and Hypotheses

We hypothesize that the combination of a simplified sewerage infrastructure and community engagement activities will reduce the risk of *Leptospira* infection among disadvantaged urban communities in Salvador, Brazil. We further hypothesize that this approach will be effective in reducing: 1) frequency of human contact with open sewerage contaminated with pathogenic *Leptospira*; 2) rat infestation; and 3) *Leptospira* load in the soil around the simplified sewerage**.** We aim to evaluate the efficacy of a sewerage intervention on reducing *Leptospira* infection and other health outcomes related to sanitation in disadvantaged urban communities in Brazil. Our specific objectives are to: (1) to evaluate prospectively the effectiveness of a simplified sewerage intervention in reducing the risk of *Leptospira* infection; (2) to determine the mechanisms by which the simplified sewerage reduce direct human contact with environmental pathogen load in urban slums. **Figure 1** provides further details on our hypotheses concerning the effects of the sanitation intervention on the transmission mechanisms of urban leptospirosis. We conceptualize this protocol as focusing on the sanitation intervention to decrease leptospirosis; however, this study protocol generates rich opportunities to evaluate other health outcomes. Therefore, we additionally aim to investigate the effects of the sewerage intervention on childhood exposure to a range of enteropathogens, and long-term child neurodevelopment and anthropometry. As exploratory outcomes, we will also investigate the effect of the sewerage intervention on the reduction of risk of arbovirus infectious diseases.

METHODS AND ANALYSIS

Overview of study design

This is an intervention trial with a cluster non-randomised controlled trial, with eight clusters (four intervention and four control), including longitudinal serological follow-up of 1,400 adults' residents from disadvantaged urban communities at high risk for leptospirosis transmission in Salvador, Bahia, Brazil. The primary outcome will be *Leptospira* infection. Each pair of intervention and control clusters will be allocated in distinct hydrological sub-basins with distances from 1,000 to 1,500 meters apart. Clusters will be matched on socio-economic status (SES), topographic or hydrologic characteristics, population density, and *Leptospira* seroprevalence. The cluster selection will also consider exposure to the pathogen by selecting houses that are located to similar distances to new sewerage system and control open sewers. The intervention aims at a combination of sanitation (simplified sewerage intervention) and community engagement activities.

Study settings

Salvador is the third largest city in Brazil with a population of 2.9 million residents [25]. Using a surveillance database of active leptospirosis in the study setting between 1996 and 2018, we performed a population-based, adjusted Kernel analysis among geo-localized records of households with leptospirosis cases to identify high risk area corresponding to 8.06% of the total area of the city [15,26]. We selected eight clusters (four intervention and four control) with average size 0.03 Km^2 located in these areas. The intervention clusters will be located in areas where the provision of simplified sewerage and community engagement is planned. Moreover, the choice of the control group is dependent on the implementer's information regarding non-intervention planned in the area for a short-term period of 5 years. Geographic location of intervention and control clusters is detailed in **Figure 2**.

Description of the Sewerage intervention

During 2022 and 2023, the sewerage intervention (simplified sewerage intervention) will be implemented by the Public Water and Sanitation Bahia State Company (*Empresa Baiana de Águas e Saneamento*) in different urban locations in Salvador, Brazil. The expansion of the sanitation coverage in the city has generated an opportunity to assess the longitudinal effectiveness of the intervention on the transmission of sanitation-related diseases, such as leptospirosis. The company is government-owned, with responsibilities for managing, monitoring, and distributing water and sanitation services throughout the state of Bahia. The costs, management, and entire execution of the work will be the responsibility of the water and sanitation company, while the team of researchers will only monitor the development of the work.

This simplified sewerage intervention is characterized by receiving the sewage generated by a cluster of houses instead of individual houses. In conventional sewerage system designs, each household is connected directly to a principal line while in the sewer simplified system households are connected to branch lines within each block [24] **(Supplementary figure 1)**.

The sanitation company will carry out the sewerage network works, which include the installation of a 150 mm primary pipe at the bottom of the residential lot, bordering a watercourse. Household sewerage networks are interconnected on one main line connecting to the main network, thus the intervention is expected to increase sanitation coverage in the study clusters by expanding the collection network. These interventions would exclusively be of a sewerage nature, with no complementary functions such as local drainage. Importantly, the intervention also includes community participation in the action designs and implementation.

Community engagement

Simplified sewerage includes different degrees of social participation. We recognize that the intervention in sanitary sewerage impacts the local environment and the communities' perception is affected by the sanitation interventions. Problems and failures related to sewerage intervention have been reported as mainly due to the difficulties in the relationship between the technicians who accompany the works and the communities which reduced the community participation to sewerage maintenance [23].

Faced with this complexity related to urban sanitation, the community engagement processes that will be implemented in this study are focused on approaches that would stimulate community mobilization to participate in the research processes, popular health education actions, and in the evaluation of the implementation of sewerage intervention. Popular Health Education (PHE) actions will be oriented toward disease prevention and health promotion of the population. The PHE is a "set of practices targeted at individuals and the community" [27,28], and this study intends to contribute to community empowerment and support for the management of sewerage intervention. The community engagement activities would be implemented in stages and with different stakeholders, including the community leaders, residents, and a multidisciplinary team of researchers including epidemiologists, ecologists, sanitation engineers, anthropologists, environmental microbiologists, geographers, economists, statisticians, etc. The study, therefore, aims to strengthen community participation in the decision-making in the management of sewerage intervention, and also support the involvement of residents in activities research such as (1) Community and institutional mobilization; (2) Collaborative mapping; and (3) Popular health education actions. Moreover, we will perform community ethnography to deepen the understanding of the social and cultural impacts caused before and after the intervention, as well as its environmental impacts and the mobilization processes.

Study population and sample size

To evaluate the effectiveness of the simplified sewerage intervention in reducing the risk of *Leptospira* infection, we plan to recruit an estimated 1,400 participants aged ≥18 years, residing in the selected clusters (approximately 175 adults per cluster in baseline). To evaluate the intervention effect on secondary (sewerage contact, environmental pathogen load, long-term child development) and exploratory (arbovirus infections, diarrheal disease, and enteric infection) outcomes, we plan to increase our sample size by 420 (30%) participants, aged between 6 months and 17 years, totaling 1,840 people. All participants from both intervention and control clusters will be followed over a period of three years after the sewerage intervention and a baseline prior to the intervention **(Figure 2)**. In the flow diagram, we provide a timeline and trial study phases in detail (**Figure 3)**.

The sample size was estimated using data on *Leptospira* infection from a previous leptospirosis cohort study in Salvador, Brazil [14]. We divided the previous leptospirosis cohort study [14] into areas of similar size to that the clusters used trial and calculated the between-cluster variation. Of 1,840 individuals participating in the previous cohort, 142 (7.9%) experienced leptospiral infection (k/ICC=0.03). Power calculations are based on a cluster-level using effect sizes (% decrease in incidence between intervention and control clusters) 40, 50, and 60%, assuming an infection rate of 0.08 per person-year. All sample size calculations used methods for matched cluster-randomized trials with the study structure of four matched pairs of clusters. Sample size estimates assumed a 12% annual drop-out rate across the three-year study period.

Control selection

Control clusters (CONT) will be selected in locations with an incomplete sewerage sanitation system. Matching criteria for control clusters include: location in a geographic area that has similar environmental (elevation, hydrologic data) and social (income, education, population density) characteristics to the intervention clusters; and at a distance of 1,000 and 1,500 meters from the intervention cluster. Areas that received major infrastructure interventions and sanitation improvements in the last five years will be excluded from the selection frame for control clusters.

Eligibility and enrolment

Before the recruitment of study participants, a census will be carried out within the selected eight clusters to enumerate all residents from households within <40m distance from an open sewer. This would ensure that households and residents living in areas with a higher risk of leptospirosis transmission are included. At each census, the individual data (name, sex, age) and the geo-coordinates a primary household respondent would be collected. As a further step, we will randomly select population-based cohorts with 95 households (175 participants) per cluster. Residents will be eligible for inclusion in the study, if (1) they are 18 years of age or above, (2) have lived in the area for at least six months, (3) sleep in the household for at least 3 nights per week, and (4) consent to the study procedures and complete an informed consent form. However, residents will be excluded if: (1) they do not meet all of the inclusion criteria; (2) they cannot provide written informed consent to participate in the study, (3) they refuse or are unable to provide demographic information and biological sample; (4) they are with limited mental health problems or other problems that have implications for survey responses or participation in any other phase of research activity.

For secondary outcomes we will include all children and young between 6 months and 17 years of age will be included in the study **(Supplementary table 1)**. We will apply similar inclusion and exclusion criteria as described but excluding children for which their legal guardians do not provide written informed consent for their participation.

Outcomes

Primary outcome measure

The study trial will consist of three specifics phases: (1) baseline; (2) sanitation intervention implementation; (3) follow-up of the residents and evaluation of the sanitation intervention **(Figure 2)**. During the phase (1) a baseline survey will be conducted to characterize the demographics, socio-economic status and *Leptospira* seroprevalence in each cluster. During the phase (3) biannual serosurveys in the cohorts will be conducted to measure the primary outcome, *Leptospira* asymptomatic infection, and estimate the risk of infection during the dry and rainy seasons. The field team will collect blood samples from eligible participants with age ≥18 years. A blood sample (10mL) will be collected from each study participant and examined for *Leptospira* antigen using the microscopic agglutination test diagnostic (MAT) [4]. MAT is the standard test to determine *Leptospira* infection in longitudinal studies and *Leptospira* infection will be defined as a four-fold rise in MAT titer or seroconversion (negative to ≥1:50) between samples from consecutive serosurveys. We will use a panel of six reference strains: representing *L. interrogans* serovars Autumnalis strain Akiyami A, Canicola strain H. Utrecht IV and Icterohaemorrhagiae strain RGA, *L. borgpetersenii* serovar Ballum strain MUS 127, *L. kirschneri* serovar Cynopteri strain 3522C and Grippothyphosa strain Duysterand the two clinical isolates: *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 (isolated locally in 1996) and *L. santarosai* serogroup Shermani strain LV3954 (isolated locally). This procedure had the same performance in identifying MAT seroconversion in our prospective studies [4,15] compared with the WHO recommended panel of 19 reference serovars. The screening will be performed with serum dilutions of 1:50-1:100. When agglutination is observed at 1:100, the sample will be titrated to determine the highest titer. As part of quality control procedures, two independent evaluations will be conducted for 8% of the samples and all samples from infected subjects.

Secondary outcomes

Environmental exposure secondary outcomes

Secondary outcomes for environmental effects will include microbiological assays to assess soil contamination with pathogenic *Leptospira*. The presence and concentration of pathogenic *Leptospira* will be evaluated in the eight clusters. Bases on prevalence the contamination soil with *Leptospira* in studies previous and packing density of 0.4 with minimum distances between collection points, a total of 68 soil samples and 15 water sewage samples will be collected in each cluster and transported refrigerated to the laboratory within six hours for processing. Sampling will take place in duplicate, with intervals of seven to nine days between them. After processing, soil and sewage samples will be stored at -80°C until molecular assays can be performed. The presence and concentration of pathogenic *Leptospira* will be evaluated using a qPCR assay (quantitative polymerase chain reaction), targeting the *lipL32* gene. Samples will be considered positive when the two qPCR replicates show amplification before a Cq of 40 [8]. Environmental variables for rainfall, temperature, landscape (remote sensing using drones), flooding, and hydrological features will also be collected during rainy seasons before and at least three months immediately after the sewerage intervention.

Rats Survey

Rat abundance will be examined at each cluster using tracking boards to quantify the level of rat activity, before and after the interventions. The use of tracking boards to quantify rat presence and activity (as rat abundance proxy) will be performed, following previously described, validated, and optimized methods [29]. Tracking boards will be distributed in each cluster for two days in a row and will be scored daily. The overall level of rat presence for each cluster will be estimated based on the percentage of positive tracking boards and the intensity of marks on each board before and at least three months immediately after the sewerage intervention.

Human movement

Environmental exposure to *Leptospira* (i.e., proximity to open sewer and unpaved roads; housing conditions; and flooding) will be quantified by studying of the individual movement of the participants in each study cluster. Movement evaluations will be performed biannually, before and after the sewerage intervention. We will focus on a sub-cohort of 320 residents living in each of the eight study clusters, divided into four groups: young males (18–34 years), young females (18–34 years), old males (aged ≥35 years), and old females (aged ≥35 years). We will use GPS data loggers that can measure small-scale movement patterns [30,31].

Forty residents in each of the study clusters will be invited to use a GPS data logger IgotU® GT-600 device (Mobile Action Technology, New Taipei City, Taiwan) [32]. Additionally, we will obtain information about movement and behavior through resident diaries and retrospective mapping before and after the intervention. Diaries will provide qualitative information on the number of hours inside/outside the study cluster and the household; contact duration and nature of the contact (i.e., contact duration with and without use of boots) with contaminated sources; and the activity performed (i.e., working, leisure, etc.) for 7 days. Retrospective mapping will provide information on the past 24 hours' pathways traveled by the residents, and contacts with contamination sources from the residents' perspective [31]. To determine participants' compliance with the study protocol using the GPS data logger and diary entry is defined as adherence for blood collection and direct observations will be conducted for participants ≥18 years of age. Non-compliance is defined as non-adherence to all study steps.

Other outcomes

Human health and wellbeing

The data will be collected pre and post-intervention to evaluate the impact on well-being between arms. Other outcomes related to individual and household health and well-being include subjective measures - self-reported general, physical and emotional well-being using the Short-Form Health Survey-12 (SF-12) [33]; perceived living conditions questions; the short version (five items) of the Brazilian Food Insecurity Scale [34] which measures the perception and experience of hunger over the past 3 months; and objectives measures - productivity through employment and income. We will also evaluate whether there will be a decrease in material and economic loss measures due to floods and landslides -which often occur in the areas without adequate sanitation.

Long-term child development

Enteric pathogens associated with diarrhea can inhibit a child's ability to absorb nutrients, further exacerbating malnutrition and developmental delays. Studies are indicating a growing association between enteric infections and environmental enteropathy, a disorder characterized by reduced intestinal barrier function, abnormal intestinal morphology, and increased inflammation [35,36]. This disorder is thought to stem from unsanitary conditions that lead to repeated exposures to enteric pathogens. Additionally, poor gut health, chronic immune stimulation and malnutrition, are linked to poor neurodevelopment in children, as well as stunting. In poverty-stricken environments, these nutritional and environmental risks can also affect the mother-child behavior patterns, which are critical for normal development, including a balance between time spent with caring their children and sanitation home issues [37].

Child neurodevelopment will be evaluated using the Ages & Stages Questionnaires 3rd edition (ASQ-3) for children aged 6 to 66 months and with the Brazilian National Assessment of School Performance (ANRESC - *Avaliação Nacional do Rendimento Escolar)* for children older than 66 months, in the pre- and post-intervention period. ASQ-3 is a tool used to monitor the child's developmental progress, it includes fine and gross motor, communication, problem-solving, and personal-social domains [38]. ANRESC is part of the Brazilian Basic Education Assessment System, which included the assessment of Math and Language skills [39]. The child development assessment will also include measurements of weight, height, BMI and head circumference. We will use the WHO Child Growth Standards and the results will be summarized using the Z-score [40].

Enteric diseases

To evaluate the impact of the intervention on enteric pathogen exposure among young children and, we will collect stool and/or rectal swab samples from children aged 6 months to 5 years old in the pre-post-intervention periods. We will use a custom-designed TaqMan Array Card

(TAC) to analyse samples for the presence of over 30 enteric pathogens, including viruses, bacteria, protozoa, and helminths. The TAC uses microfluidic technology to simultaneously perform 48 individual quantitative polymerase chain reactions per sample and has been previously validated and used for detection enteric pathogens in stool [41–43].

Arboviral diseases

We will evaluate the effectiveness of the sewerage intervention and the changes in landscape characteristics associated with them, in the spatiotemporal distribution of arbovirus (Dengue, Zika, Chikungunya) vector mosquitoes. We will carry out entomological investigations in private (participants' homes) and public areas with the collection of adult and immature mosquitoes by aspiration [44], with the verification of identified mosquitoes breeding sites, to measure their density. We will verify whether there is a spatiotemporal pattern in the presence and density of vector mosquitoes in the sampled regions, to allow us to investigate the possible factors that may be related to changes in the distribution of exposures before, during, and after the intervention process and whether these interventions have modified ecological indices for vector abundance.

Data Collection and management

Trained interviewers will administer specific questionnaires to participants through face-to-face interviews to collect general demographic information, socio-economic data, exposure factors and individual or collective sanitation practices, and knowledge about leptospirosis and other diseases. In the case of children at home, a new questionnaire will be answered by the caregiver. Information will be collected on the child's general health status, development, and medical history and diseases **(Supplementary table 2)**. The questionnaires and all information will be confidential and coded using individually identifiable numeric identifiers. Every effort will be made to protect the privacy of the study participants and all data collected will be treated as confidential.

All questioners and forms described in this protocol will be included in the Research Electronic Data Capture (REDCap) database. The REDCap web version as well as its mobile application will be used to record all data using mobile devices. A data management staff will keep track of the information in real time.

Participants and public involvement

All study subjects will be involved in the trial, answering questionnaires, providing biological samples, and involved in engagement activities as previously described in the section on community engagement. The sanitation intervention will be conducted by a governmental agency, and the implementation of this intervention involves dialogue with the residents of the communities. However, the study design and conduct of the trial of this intervention study will be taking place independently of public involvement. After each follow-up completion, research findings will be shared and disseminated in a group's meetings or visit households with key stakeholders.

Statistical analysis

Descriptive statistics will be employed to report the demographic, social, economic, or environmental characteristics between the study clusters (intervention and control clusters). For the primary outcome, we will apply quasi-Poisson regression to estimate the incidence rate ratio (IRR) of *Leptospira* infection. We will also evaluate the effect of the intervention on *Leptospira* infection risk using mixed-effects logistic regression models to account for clustering of the observations within study units and across repeated measures for individuals. We will model the overall effect of the intervention on infection risk while controlling for baseline sewerage and flooding exposure and potential confounding variables, such as age, sex, indicators of the socio-economic level, and rainfall intensity during an annual period, which are presumably unrelated to the intervention **(Figure 1)**.

For the secondary outcome, we will conduct environmental analyses of *Leptospira* load between intervention and control clusters to compare environmental risk. To do this, we will model two leptospiral load outcomes, prevalence (binomial) and concentration (log-transformed), using generalized linear models with random effects to account for clustering within study units. The association between environmental contamination and hypothesized predictors of risk (rat abundance, human movement, and ecological data) will also be explored in the model **(Figure 1)**.

Limitations and bias

The allocation is non-randomised with selection based on intervention criteria determined by the implementer so the risk of residual confounding cannot be excluded. The choice of the control group is also dependent on the implementer's information about the non-intervention in the area in the short term (5 years-period). To limit the potential for bias arising from our non-randomised design, we will select control group with as similar as possible socio-economic and environmental characteristics to those of their matched intervention clusters.

We will perform the impact calculation through the double differentiation of the averages of the results found between the intervention and control clusters before and after the sewerage intervention. The mitigation of bias will be carried out through the control of fixed effects of time and location between different intervention and control clusters over the years of evaluation.

ETHICS AND DISSEMINATION

This project was evaluated and approved by a local ethics committee (CEP/ISC/UFBA) under number CAEE 32361820.7.0000.5030, and a national research ethics committee (CONEP) linked to the Brazilian Ministry of the Health under approval number 4.235.251. The trial study protocol was registered with the Brazilian Clinical Trials Registry, RCT trial RBR-8cjjpgm (https://ensaiosclinicos.gov.br/rg/RBR-8cjjpgm). All participants involved in the study will provide informed consent before any study activity, and the community leaders, previously identified by the team, will provide verbal consent before enrollment of the community in the trial. Participants aged 12 and 17 will sign an assent form, in addition to a participation consent form completed through their parents or legal guardians. Children under the age of 12 will have their consent signed by their parents or legal guardians only. The results of the trial will be presented at local and international meetings and submitted to peer-reviewed journals for publication. Results will also be shared directly with the participating communities. All individual information participants will be kept and published anonymously.

Authors' contributions

FC, CC, MGR, AK, YAL, PD, CS and OC contributed with project's main conceptual ideas; FC, ME, EG, and CC contributed to study design and statistical analysis plan; FNS, DO, FAP, CS, JFA, PSR, JOS, RHC, DS, RN, RLB, VAM, JPAT, JK, PR, FC, and CC developed field and laboratory protocols and implementation; FNS, FAP, CC, RHC, and FC developed data collection tools; FNS, FAP, JFA, RHC, PR, CC, and FC contributed to drafting the main manuscript. All authors contributed to editing and revising the manuscript.

Funding statement

This work was supported by Wellcome Trust grant number 102330/Z/13/Z. All project costs are jointly funded by Wellcome Trust and the Department of Health and Social Assistance

(DHSC), through the National Institute for Health Research (NIHR), using the UK Official Development Assistance Fund (ODA).

Competing interest statement

Not applicable

Data sharing statement

The data collected with personal identifying data redacted will be available encoded for use by principal investigators on request following the publication of trial results. The funders have no role in the study design, data collection, and analysis, decision to publish, or preparation of manuscripts.

Acknowledgments

The authors gratefully acknowledge critical input on the study protocol and all the study participants. We acknowledge the partner institutions for their collaboration and dedication. We thank Patricia Lustosa, and Mayara Carvalho for their help with the review and discussion of this protocol, Ana Maria Silva and George G. Machado for help with supplementary figure, and Hammed O. Mogaji for his help with manuscript proofing

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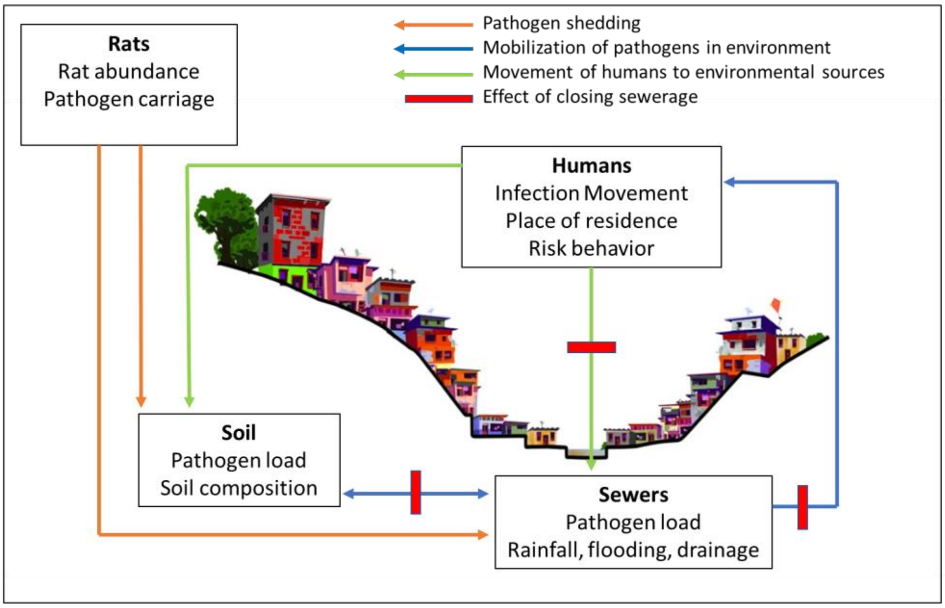
FIGURES

Figure 1- Rationale, hypothesis, and outcomes assessed in the study. We hypothesize that sanitation intervention will improve the environmental conditions and change the behavior of humans, resulting in both direct and indirect impacts on the incidence of leptospirosis and other diseases human and well-being. * HoH- Household-level

Figure 2- Study clusters in the city of Salvador, Brazil. Altitude and sampling clusters (**A**). Example of baseline sanitation conditions of the study cluster (**B).** In (**C)** Consort flow diagram by intervention and control cluster, data collection times and type of activities.

Figure 3- Overview of the study procedures, the timeline for study, and data collection. **Note 1**: Each cluster of the study will be formed by a cluster with intervention vs. Control. The period of activities for each serological survey will take place at the time described in the figure (Allocation, Recruitment, and Baseline). **Note 2**: Update years 1 to 5 will be realized after receipt of the water and sanitation company schedule. SES: Socio-economic status.

Figure 1



Measurements	
Ascertainment	**Measure**
<u>Cluster-level</u>	
Nat. Meteorol. Serv	Weather
Remote sensing survey	Land classification
GIS survey	Topology
	Hydrology, flood-risk
Environmental surveys	Open drainage
	Open refuse deposits
Intervention Company Database	Sewerage connections
Tracking board surveys	Rat abundance
Soil *lip*L32 qPCR survey	*Leptospira* DNA load
<u>Household-level</u>	
Interviews of HoH	Socio-economic Status
Environmental surveys	Informal sewerage
	Sewage line condition
Rodent infestation survey	Infestation score
Census	Domestic animals
<u>Individual-level</u>	
Engagement Communitarian	Community participation level
Interviews	Demographics
	Risk behaviours
	Time in cluster
Serosurveys	*Leptospira* infection
GPS tracking survey	Time in proximity to sources of contamination

Figure 2

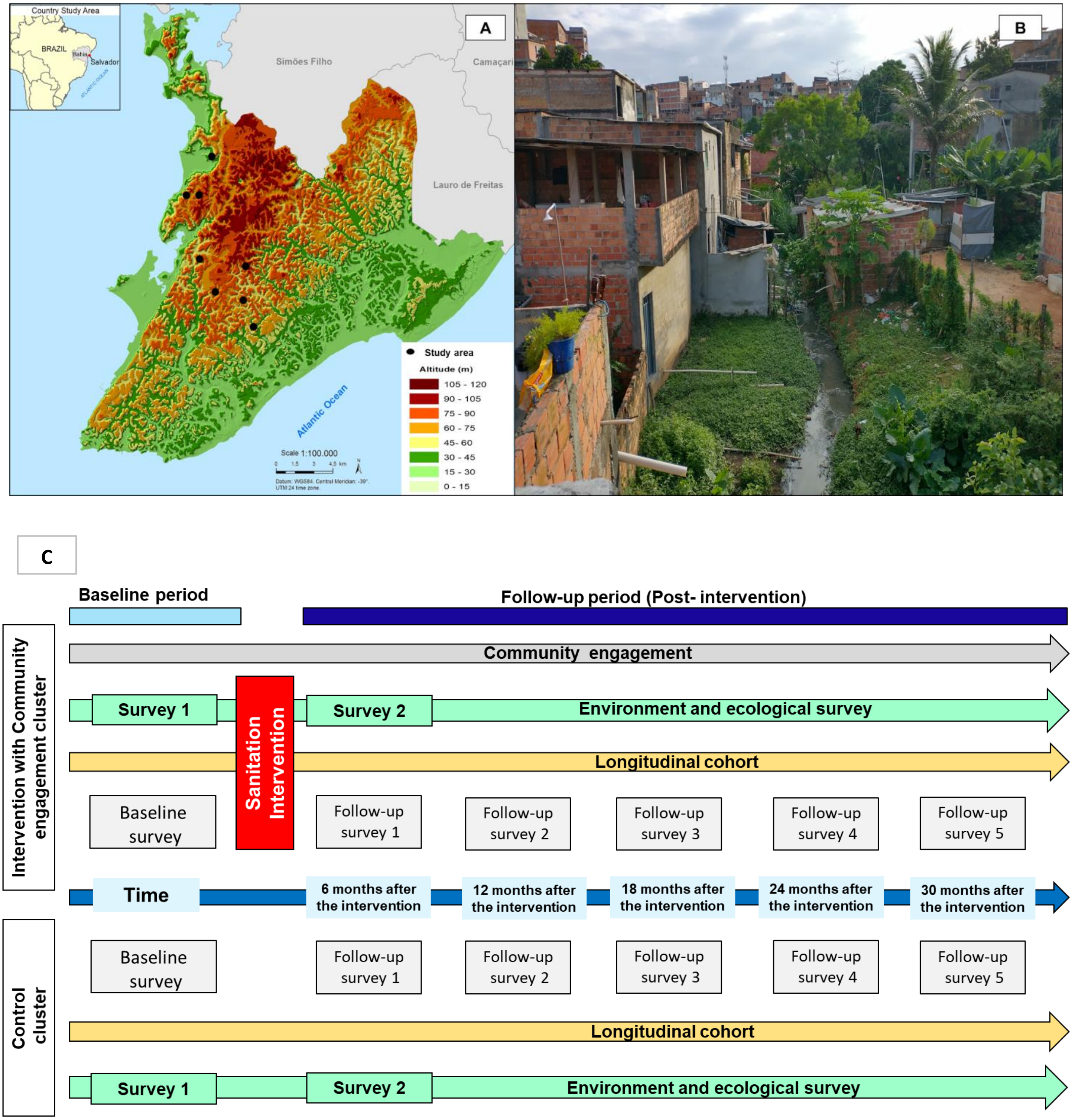
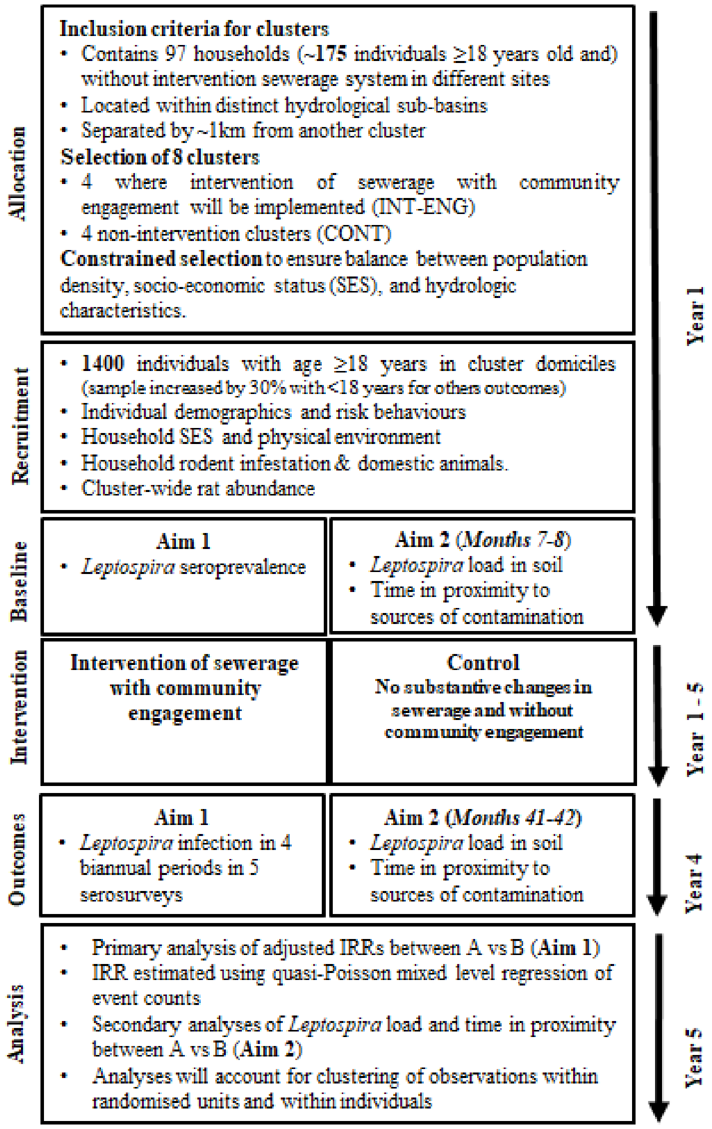

A
Country Study Area
BRAZIL
Bahia
Salvador
Simões Filho
Camaçari
Lauro de Freitas
Atlantic Ocean
Study area
Altitude (m)
105 - 120
90 - 105
75 - 90
60 - 75
45- 60
30 - 45
15 - 30
0 - 15
Scale 1:100.000
B
C
Baseline period
Follow-up period (Post- intervention)
Intervention with Community engagement cluster
Community engagement
Survey 1
Sanitation Intervention
Survey 2
Environment and ecological survey
Longitudinal cohort
Baseline survey
Follow-up survey 1
Follow-up survey 2
Follow-up survey 3
Follow-up survey 4
Follow-up survey 5
Time
6 months after the intervention
12 months after the intervention
18 months after the intervention
24 months after the intervention
30 months after the intervention
Control cluster
Baseline survey
Follow-up survey 1
Follow-up survey 2
Follow-up survey 3
Follow-up survey 4
Follow-up survey 5
Longitudinal cohort
Survey 1
Survey 2
Environment and ecological survey

Figure 3


Allocation
Inclusion criteria for clusters
• Contains 97 households (~175 individuals ≥18 years old and) without intervention sewerage system in different sites
• Located within distinct hydrological sub-basins
• Separated by ~1km from another cluster
Selection of 8 clusters
• 4 where intervention of sewerage with community engagement will be implemented (INT-ENG)
• 4 non-intervention clusters (CONT)
Constrained selection to ensure balance between population density, socio-economic status (SES), and hydrologic characteristics.
Recruitment
• 1400 individuals with age ≥18 years in cluster domiciles (sample increased by 30% with <18 years for others outcomes)
• Individual demographics and risk behaviours
• Household SES and physical environment
• Household rodent infestation & domestic animals.
• Cluster-wide rat abundance
Baseline
Aim 1
• Leptospira seroprevalence
Aim 2 (Months 7-8)
• Leptospira load in soil
• Time in proximity to sources of contamination
Year 1
Intervention
Intervention of sewerage with community engagement
Control
No substantive changes in sewerage and without community engagement
Year 1 - 5
Outcomes
Aim 1
• Leptospira infection in 4 biannual periods in 5 serosurveys
Aim 2 (Months 41-42)
• Leptospira load in soil
• Time in proximity to sources of contamination
Year 4
Analysis
• Primary analysis of adjusted IRRs between A vs B (Aim 1)
• IRR estimated using quasi-Poisson mixed level regression of event counts
• Secondary analyses of Leptospira load and time in proximity between A vs B (Aim 2)
• Analyses will account for clustering of observations within randomised units and within individuals
Year 5

Supplementary material

Supplementary Table 1. Specific age range for each outcome.

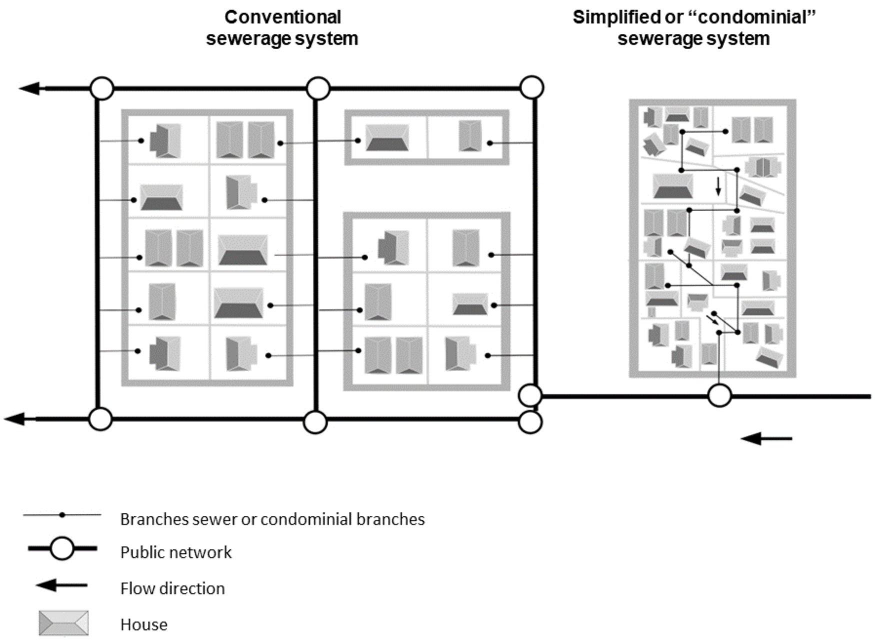
Outcome	Age group
Leptospirosis, preventive practice, well-being, perceptions of residents	≥ 18 years
Arboviral diseases	≥ 6 months
Long-term infant development	≥ 6 to ≤ 66 months
Enteric diseases	≥ 6 to ≤ 66 months

Supplementary Table 2. Primary and secondary outcomes baseline in assessed in this study protocol, Salvador, Brazil, 2022

Outcomes of interest	Operational definition
1. Individual *Leptospira* prevalence	
	> *Leptospira* antigen in a blood sample using microscopic agglutination test diagnostic
2. Individual risk for *Leptospira* transmission	
	> Walk-in flooding in the neighborhood
	> Walk barefoot outside the household
	> Need to unclog sewage
	> Perception of vulnerability to leptospirosis
	> Perception of the severity of the leptospirosis disease
	> Perception of the presence of rats near the house
3. Environmental for *Leptospira* transmission	
	> Proximity and contact frequency to open sewer, trash, and other exposures to environmental sources
	> Sewage infrastructure at home and in the community
	> Flooding in the community and inside the home
	> Floor and road conditions
	> Accumulation of garbage near the house
	> Presence and concentration of *Leptospira* load in the soil
4. Sanitation-related knowledge and practice	
	Consequences of lack or poor sanitation as:
	> Structural problems
	> Major related diseases
	> Institutions responsible for providing sanitation in the community
	> Practice related to sanitation in-home and community
5. Social Capital	
	> Self-Assessment of coexistence and trust with neighbors.
	> Perception of neighbors' willingness to help each other
	> Social, leisure, and community support activities practiced
	> Willingness to continue living in the community
6. Well-being	
	Well-being will be evaluated through measures such as:
	> Productivity through the assessment of income and employment
	> Quality of health life (SF-12 questionnaire).
	> Food insecurity (EBIA-5 questionnaire)
	> Loss of goods, services, and income due to flooding and landslides
7. Long-term child development	
	> Global and specific development in gross and fine motor coordination, communication, problem-solving and personal-social domains (ASQ-3 questionnaire)
	> Performance evaluation in Portuguese and Mathematics tests of Brazilian elementary education (Andres)
	> Anthropometric measures
8. Enteric diseases	
	The self-reported gastrointestinal infection will be evaluated through caregiver-reported symptom data in the last 7 days including:

	> Diarrhoea (≥3 loose or liquid stools in a 24h period)
	> Vomiting
	> Abdominal pain
	> Refusal to eat
	> Hygiene practices
9. Arboviral diseases	
	> Accumulation of water in containers after rain
	> Presence of mosquitoes at home
	> Main time of the presence of mosquitoes at home
	> Diagnosis of an arbovirus in the last 6 months

Supplementary Figure 1. Schematic layout of the design of the conventional and simplified sewerage systems.



Capítulo IV (Short Communication)

Artigo publicado no periódico *Vector borne and zoonotic diseases*

Fator de impacto: 1.333 (2021); Qualis A2 (Saúde Coletiva)

DOI: 10.1089/vbz.2020.2686

Increased rat sightings in urban slums during the COVID-19 pandemic and the risk for rat-borne zoonoses

Increased rat sightings in urban slums during the COVID-19 pandemic and the risk for rat-borne zoonoses

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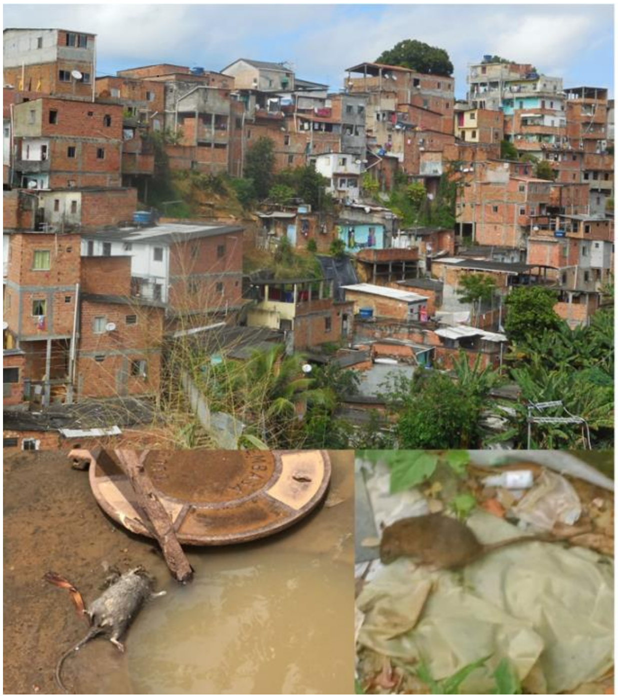
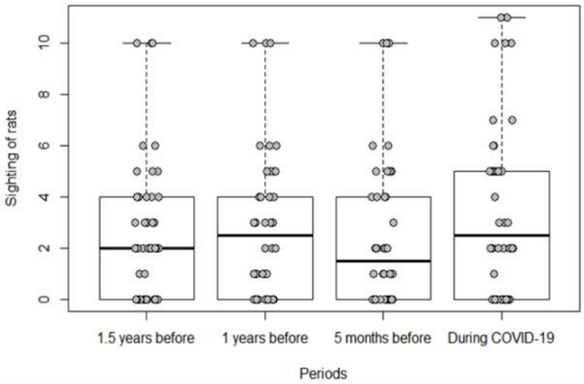
Keywords: COVID-19 pandemic; urban slums; rats

Social isolation has been implemented in many countries around the world to reduce the impact of COVID-19 during the 2020 pandemic. Although, this measure is critical in preventing the spread of the virus, the initiative has lead to rodent-human behavioural changes, resulting in health, economic and social impacts (Corburn et al., 2020; Heymann & Shindo, 2020; Battersby et al., 2008). Globally, an increase in rodent related problems has been reported in various media outlets by experts during the pandemic. In low and middle income countries (LMIC) such as Brazil, where a large proportion of urban residents live in slum communities, with substandard water, sanitation and hygiene coverage, social isolation seems to have provided an ecological opportunity for rodents' expansion, and any increase in the population of rodents during the social isolation could aggravate the risk of zoonoses among vulnerable residents, since rodents are reservoirs of several zoonotic diseases (Battersby 2015).

Figure 1. Distribution of the number of sighted rats among residents of an urban community in Brazil (Sanitary District of Pau da Lima, Salvador-Brazil) and the effect of social isolation during the COVID-19 pandemic on the sighting of rats inside urban slum spaces.





	Sighting of rats during the Covid-19 pandemic		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	1.95	1.43 – 2.66	**<0.001**
1.5 years before (Ref.)	-	-	-
1 years before	1.04	0.82 – 1.33	0.749
5 months before	0.95	0.74 – 1.22	0.685
During COVID-19	**1.27**	**1.00 – 1.60**	**0.045**
Observations	184		

We collected data on rat sightings from consenting participants during home visits in a slum community-based longitudinal study on urban leptospirosis in Salvador, Brazil. Participants were interviewed over more than two years in three biannual follow-ups. Then, out of the 287 residents participating in the cohort during 2018-2019, 46 answered the same questionnaire via an instant messaging application three months after the commencement of social isolation (June 2020) (Fig 1). Their responses were included in a mixed effects model to compare the number of rat sightings at different time periods. These periods were grouped as 18 months, 12 months and 5 months before COVID-19, plus the COVID-19 sample, which was temporally equivalent to the first pre-COVID sample. Rat sightings were 123, 128, 114 (all pre-COVID) and 156, respectively. The perceived increase in the number of rats seen by residents during the social isolation, representing 1.27 (CI: 1.00-1.60) in the number of rats sightings, the most among the different time periods (before and during COVID-19) in Brazil (Fig. 1). There was, however, no difference in the proportion of people who saw rats across the periods.

The closing of trade reduced trash collection (that was already precarious) and an increase in the number of residents living in small spaces may all have contributed to the increase in the accumulation of garbage, a source of food for rodents. The increase in rat activity may in turn increase residents' exposure and susceptibility to diseases, for example toxoplasmosis, leptospirosis, salmonellosis, diarrhea and viruses like haemorrhagic fever with pulmonary syndrome, haemorrhagic fever with renal syndrome, Lassa fever among others through elevated rodent-human contact and subsequent disease transmission. In a similar vein, social isolation is coinciding with arbovirus epidemics in the same city, suggesting environmental changes and human behavioural changes that may influence both vectors and reservoirs of disease (Ribeiro et al., 2020),

The COVID-19 pandemic has brought about changes in human behaviour likewise increase in inequality and vulnerability of residents in poor urban communities. Above all, the health authorities have to target multiple efforts that go beyond control actions for the prevention of COVID-19, since these populations live in a scenario of increased exposure and risk to multiple diseases in this pandemic.

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Corburn, J., Vlahov, D., Mberu, B., Riley, L., Caiaffa, W. T., Rashid, S. F., Ko, A., Patel, S., Jukur, S., Martínez-Herrera, E., Jayasinghe, S., Agarwal, S., Nguendo-Yongsi, B., Weru, J., Ouma, S., Edmundo, K., Oni, T., & Ayad, H. (2020). Slum Health: Arresting

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Ribeiro, V. S. T., Telles, J. P., & Tuon, F. F. (2020). Arboviral diseases and COVID-19 in Brazil: Concerns regarding climatic, sanitation, and endemic scenario. *Journal of Medical Virology*. https://doi.org/10.1002/jmv.26079

CONCLUSÃO E CONSIDERACOES FINAIS

A presente tese de doutorado explorou os efeitos de intervenções sanitárias nos diferentes aspectos da dinâmica de transmissão da leptospirose em comunidades urbanas vulnerabilizadas de Salvador, Bahia, Brasil. Neste estudo integramos dados ecológicos e epidemiológicos a fim de caracterizar o papel de obras sanitárias na manutenção dos mecanismos de transmissão de doenças, concentrando novas evidências e avançando na compressão com relação a estudos anteriores limitados. A seguir, são apresentadas as conclusões específicas referentes aos capítulos contidos nesta tese.

No primeiro capítulo concluímos que intervenções urbanas de canalização e tamponamento dos corpos d`água poluídos com esgotos domésticos reduzem a presença de ratos, mas não contribuem na redução da incidência de *Leptospira*. Porém, nossos achados, no capítulo dois, mostraram que as intervenções governamentais na rede de esgotos diminuem a contaminação dos solos, diminuindo a concentração e prevalência da bactéria *Leptospira* no ambiente. No terceiro capítulo, informamos a importância da inclusão do envolvimento e engajamento dos usuários do saneamento nas estratégias e planejamento de intervenções sanitárias e em protocolos de pesquisa de avaliação de impacto de intervenc sanitárias . Neste capítulo, elaboramos um *framework* teórico-prático para avaliar o impacto destas intervenções sanitárias com participação social para reduzir os níveis de exposição, proporcionando efeitos positivos e duradouros na saúde das populações. Por fim, no capítulo quatro, relatamos o aumento no avistamento de ratos pelos residentes de comunidade carente durante a pandemia de Covid-19, concluímos que as restrições pandêmicas, fechamento do comércio, redução dos serviços de limpeza urbana e aumento das densidades domiciliares podem ter contribuído positivamente as populações de ratos locais.

De maneira geral, as principais conclusões que podemos citar desta tese são:

1. Intervenções em saneamento continua sendo uma necessidade urgente nas comunidades urbanas empobrecidas;
2. Este é o primeiro conjunto de estudos que avaliam o impacto de intervenção governamentais e comunitárias de esgotamento na tríade da dinâmica de transmissão de leptospirose urbana (roedores, ambiente e exposição humana);
3. Condições socioeconômicas precárias, a onipresença das fontes de contaminação da bactéria *Leptospira* e elevada incidência da doença nas comunidades carentes limita os efeitos das intervenções. Faz-se necessário intervenções abrangentes e integradas de

saneamento, se possível com participação da comunidade para obter resultados positivos e duradouros.

4. A implementação de obras de esgotamento e infraestrutura devem considerar como meta reduzir a carga de doenças, a fim de interromper todas as vias de transmissão: reservatórios zoonóticos, contaminação do ambiente e exposição humana.
5. É de suma importância estudos interdisciplinares na resolução de problemas relacionadas ao WASH (*Water, Sanitation and Hygiene*), reduzindo a carga de doenças e as iniquidades e disparidades sociais que acometem a populações carentes em todo o mundo.
6. Salientamos, a importância das ciências ecológicas e sua interface com a epidemiologia na redução de impactos das doenças zoonóticas e ambientais emergentes e reemergentes nas populações residentes em comunidades carentes, assegurando entender os mecanismos e fatores que contribuem para o controle e redução de incidência destas doenças.

ANEXOS

Trabalhos publicados em colaborações durante o período do doutorado. Foram um total de 10 artigos publicados nos quais sou autor e coautor, estes trabalhos estão listados nos anexos 1 ao 10.

Anexo 1: A multivariate geostatistical framework for combining multiple indices of abundance for disease vectors and reservoirs: a case study of rattiness in a low-income urban Brazilian community.
DOI: 10.1098/rsif.2020.0398

INTERFACE

royalsocietypublishing.org/journal/rsif

Research

Cite this article: Eyre MT *et al.* 2020 A multivariate geostatistical framework for combining multiple indices of abundance for disease vectors and reservoirs: a case study of *rattiness* in a low-income urban Brazilian community. *J. R. Soc. Interface* **17**: 20200398. http://dx.doi.org/10.1098/rsif.2020.0398

Received: 27 May 2020
Accepted: 6 August 2020

Subject Category:
Life Sciences–Mathematics interface

Subject Areas:
environmental science

Keywords:
epidemiology, abundance indices, zoonotic and vector-borne diseases, multivariate model-based geostatistics, leptospirosis, Norway rat

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5096322.

THE ROYAL SOCIETY PUBLISHING

A multivariate geostatistical framework for combining multiple indices of abundance for disease vectors and reservoirs: a case study of *rattiness* in a low-income urban Brazilian community

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A key requirement in studies of endemic vector-borne or zoonotic disease is an estimate of the spatial variation in vector or reservoir host abundance. For many vector species, multiple indices of abundance are available, but current approaches to choosing between or combining these indices do not fully exploit the potential inferential benefits that might accrue from modelling their joint spatial distribution. Here, we develop a class of multivariate generalized linear geostatistical models for multiple indices of abundance. We illustrate this novel methodology with a case study on Norway rats in a low-income urban Brazilian community, where rat abundance is a likely risk factor for human leptospirosis. We combine three indices of rat abundance to draw predictive inferences on a spatially continuous latent process, *rattiness*, that acts as a proxy for abundance. We show how to explore the association between *rattiness* and spatially varying environmental factors, evaluate the relative importance of each of the three contributing indices and assess the presence of residual, unexplained spatial variation, and identify *rattiness* hotspots. The proposed methodology is applicable more generally as a tool for understanding the role of vector or reservoir host abundance in predicting spatial variation in the risk of human disease.

1. Introduction

In studies of endemic vector-borne and zoonotic diseases, estimates of vector and reservoir host abundances, including spatial variation in abundance, are often needed to inform predictive models of disease risk and to guide the decision-making process for the implementation, monitoring and evaluation of control programmes [1]. Detecting all members of a target population at a sampled location is impossible for most disease vector or reservoir species.

Anexo 2 (Short paper): Prevalence of Diarrheagenic *Escherichia coli* (DEC) and *Salmonella* spp. with zoonotic potential in urban rats in Salvador, Brazil.

DOI: 10.1017/S095026882000285X

Epidemiology and Infection

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Prevalence of Diarrheagenic *Escherichia coli* (DEC) and *Salmonella* spp. with zoonotic potential in urban rats in Salvador, Brazil

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Short Paper

Cite this article: Pimentel Sobrinho C *et al.* (2021). Prevalence of Diarrheagenic *Escherichia coli* (DEC) and *Salmonella* spp. with zoonotic potential in urban rats in Salvador, Brazil. *Epidemiology and Infection* **149**, e128, 1–4. https://doi.org/10.1017/S095026882000285X

Received: 4 March 2020
Revised: 15 July 2020
Accepted: 16 September 2020

Key words:
Enterobacteria; *R. norvegicus*; *R. rattus*; odents; Zoonoses

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Abstract

Studies evaluating the occurrence of enteropathogenic bacteria in urban rats (*Rattus* spp.) are scarce worldwide, specifically in the urban environments of tropical countries. This study aims to estimate the prevalence of diarrhoeagenic *Escherichia coli* (DEC) and *Salmonella* spp. with zoonotic potential in urban slum environments. We trapped rats between April and June 2018 in Salvador, Brazil. We collected rectal swabs from *Rattus* spp., and cultured for *E. coli* and *Salmonella* spp., and screened *E. coli* isolates by polymerase chain reaction to identify pathotypes. *E. coli* were found in 70% of *Rattus norvegicus* and were found in four *Rattus rattus*. DEC were isolated in 31.3% of the 67 brown rats (*R. norvegicus*). The pathotypes detected more frequently were shiga toxin *E. coli* in 11.9%, followed by atypical enteropathogenic *E. coli* in 10.4% and enteroinvasive *E. coli* in 4.5%. From the five black rats (*R. rattus*), two presented DEC. *Salmonella enterica* was found in only one (1.4%) of 67 *R. norvegicus*. Our findings indicate that both *R. norvegicus* and *R. rattus* are host of DEC and, at lower prevalence, *S. enterica*, highlighting the importance of rodents as potential sources of pathogenic agents for humans.

Synanthropic rodents of the species *Rattus norvegicus* (urban brown rat) and *Rattus rattus* (black rat) are of great importance in public health for being reservoirs of a diversity of pathogens [1]. They are hosts of *Leptospira interrogans*, *Streptobacillus moniliformis* and Seoul virus (SEOV) [1]. Moreover, they carry ectoparasites such as fleas, which act as reservoirs of *Yersinia pestis*, *Rickettsia typhi* and *Bartonella* spp. [1, 2]. All those microorganisms cause diseases of great public health importance, where humans become infected via direct contact, food or environmental contamination [1].

In temperate regions, studies have identified enterobacteria of clinical importance, such as *Salmonella* spp. and *Escherichia coli*, in these rodent species, which can be eliminated through faeces and be another source of infection for humans [3, 4]. Urban rats are likely to acquire such bacteria from the environment [4]. Identical antibiotic-resistant and virulent *E. coli* strains were found in samples from rodents captured in agricultural facilities, in domestic animals and environmental samples from the same facilities [3]. Moreover, rats living on commercial farms also carry the same *Salmonella* spp. strains that are detected in resident chickens [5].

Diarrheagenic *E. coli* (DEC) and *Salmonella* are important sources of foodborne diseases and gastroenteritis in humans [4]. *Salmonella* was the aetiologic agent most prevalent (92.2%) in 12 503 foodborne disease outbreaks in Brazil, as reported by the Information System for Notifiable Diseases (SINAN) from 2000 to 2017 [6]. Herein, we (a) estimate the prevalence of DEC and *Salmonella* in *R. norvegicus* and *R. rattus* from urban tropical slums in the city of Salvador, Brazil, determine the susceptibility profile of *Salmonella* isolates to antimicrobials, and identify *E. coli* pathotypes isolated from urban rats faeces.

We live-trapped *R. norvegicus* and *R. rattus* in four slum communities within the Suburban Sanitary District of the city of Salvador, Brazil, from April to June 2018. Approximately 30% of the populations of Salvador (and Brazil) reside in similar low-income and poor environmental conditions [7]. The sampled areas ranged from 0.07 to 0.09 km^2. Within each community, 40 randomised points were selected as trapping points, in which two Tomahawk traps were set with fresh sausage for four nights and checked early morning. Traps with individuals of

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Anexo 3 (Short Communication): Effect of chemical and sanitary intervention on rat sightings in urban communities of New Providence, the Bahamas

DOI: 10.1007/s42452-021-04459-x

Short Communication

Effect of chemical and sanitary intervention on rat sightings in urban communities of New Providence, the Bahamas

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Received: 26 October 2020 / Accepted: 1 March 2021 / Published online: 24 March 2021

Abstract

Rats are invasive pest species that commonly infest low-income urban environments. Their association with humans constitutes a threat of rodent-borne disease transmission. We evaluated the outcome of a chemical and sanitary intervention on rat sightings in seven low-income urban settlements of New Providence, the Bahamas. The intervention consisted of rodenticide application, education about environmental sanitation, and improvement in waste disposal. Rat sightings were systematically recorded by trained staff before and three months after the intervention. The intervention slightly decreased rat sightings, with an average of 2.7-fold with varied effectiveness across locations. Four out of seven locations (57%) registered a decrease in rat sightings. Our results suggest that social and environmental differences among communities may be responsible for the mixed efficacy observed in the current rodent management practice in urban communities of the Bahamas. However, a new set of control measures needs to be developed for areas where rodent decline was not observed. This study provides novel data on how rat population behaves post-intervention in a unique ecological setting like the Bahamas, presenting an informed judgment for their management, especially in the event of a natural disaster.

Keywords Rat · Rat sightings · Rodenticide · Rodent control · Urban community · Zoonoses

1 Introduction

Rats (genus *Rattus*) are widely distributed and successful invasive species that are present in all continents except Antarctica [1]. They were likely introduced into North America in the 1750s through trans-Atlantic navigation during the European colonization [2]. They adapt well to human-altered environments and are regarded as notorious agricultural and urban pests [3]. In urban settings, their infestation is often associated with low socioeconomic status, inefficient waste management, open sewers, overcrowding, and other infrastructure characteristics that can be exploited for food and harbourage [4].

Frequent rat sightings have been reported as an indicator of poor sanitation and diseases [5]. Rats are famous reservoirs of several infectious diseases (e.g.

Adedayo M. Awoniyi and Andrew Thompson have contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42452-021-04459-x.

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SN Applied Sciences (2021) 3:495 | https://doi.org/10.1007/s42452-021-04459-x

SN Applied Sciences
A SPRINGER NATURE journal

Anexo 4: Knowledge, Attitude, and Practices regarding Leptospirosis among Visitors to a Recreational Forest in Malaysia.

DOI: 10.4269/ajtmh.20-0306

Am. J. Trop. Med. Hyg., 104(4), 2021, pp. 1290–1296
doi:10.4269/ajtmh.20-0306

Knowledge, Attitude, and Practices regarding Leptospirosis among Visitors to a Recreational Forest in Malaysia

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Abstract. Leptospirosis is a zoonotic disease and a worldwide public health problem that affects mainly high-risk groups. Characterizing knowledge, attitude, and practice (KAP) among high-risk groups is important to develop appropriate prevention programs. Here, we performed a cross-sectional study among 300 visitors of a recreational forest in Malaysia to examine leptospirosis KAP and demographics. These variables were integrated to create knowledge and practice scores for each respondent. All respondents had heard about leptospirosis, and 87% of them correctly identified it as a disease. The majority of respondents had high knowledge (63%), positive attitude, and good practice (68%) toward prevention of the disease. However, there were gaps in knowledge, with 78% of the respondents indicating eating without washing hands as the major cause of leptospirosis transmission. Our final model identified that higher knowledge score was associated with higher practice score. Our results indicate that it is important to increase knowledge, especially on transmission routes of leptospirosis, among visitors in recreational areas. Moreover, more attention needs to be paid to promote good practice habits among visitors, targeting those at higher risk of being infected by leptospirosis to prevent potential outbreaks in the recreational areas.

INTRODUCTION

Leptospirosis is a globally distributed zoonotic disease with more than one million cases and 50,000 deaths annually.[1] Leptospirosis shows a range of symptoms in humans such as high fever, headache, jaundice, renal failure, meningitis, hemorrhagic fever, abdominal pain, and muscle aches; however, most infections are subclinical.[2] Leptospirosis is caused by pathogenic spirochetes from the genus *Leptospira*. Pathogenic *Leptospira* spp. infect and colonize the kidneys of almost all mammalian species[3] and are excreted at high concentrations[4,5] into the environment where they can survive for extended time.[6] Small mammals, and specifically rodents, are chronic carriers of *Leptospira* that act as the major reservoir host in the transmission of leptospirosis.[3] Infection occurs either through direct contact of broken skin, abrasion, or mucous membranes with urine or tissue of infected animal, or indirect contact with contaminated soil and water.[7] Leptospirosis is highly prevalent in the Asia Pacific region, and specifically in Southeast Asia and Oceania.[1,3] The estimated incidence of leptospirosis ranges from 0.1 to 1 for every 100,000 per year in temperate climates, but it can be as high as 10–100 per 100,000 in tropical regions, and more than 100 per 100,000 among high-risk groups during outbreaks.[8] The high prevalence of leptospirosis in Southeast Asia has been related to flooding, recreational activities, occupational exposure, travel to endemic regions, poor sanitation, and waste disposal.[3]

Leptospirosis is endemic in Malaysia, and the first case was reported in 1925.[9] A total of 12,325 cases with 338 deaths were reported between 2004 and 2012.[10] According to the Ministry of Health Malaysia,[11] Terengganu was among the five states in Malaysia with the highest number of leptospirosis cases (≈8.00 per 100,000 population), and recreational areas were categorized as one of the important hotspots of leptospirosis outbreaks. In Malaysia, most recreational areas are equipped with many infrastructures such as community halls, chalets, public toilets, and food stalls.[12] However, poor sanitation and waste disposal make recreational areas favorable environments for the transmission of leptospirosis. The presence of pathogenic *Leptospira* has been previously reported in two recreational areas in Terengganu, Lata Tembakah Waterfall and Lata Belatan Waterfall, with 5% (1/20 water and soil samples) and 10% (2/20 water and soil samples) positive samples, respectively.[13] Several factors have been identified as contributing to the increasing number of outbreak, including water pH, temperature, soil moisture, and the presence of wild animals which act as reservoirs.[13,14] *Rattus tiomanicus* was found to be the dominant carrier with 88.1% prevalence (37/42 leptospiral isolates) for serovars Icterohaemorrhagiae, Canicola, Ballum, Pyrogenes, and Hebdomadis.[15] Serovar hebdomadis was also reported as the dominant serovar in Terengganu.[14]

Despite the increasing incidence, leptospirosis is often neglected, and there is still a lack of awareness and basic knowledge on the disease, especially among high-risk groups.[16] Understanding the knowledge of individual subjects and their attitudes with respect to risky behaviors is key to implementing better prevention practices. Similarly, it is also important to identify the sociodemographic characteristics that influence individual behaviors and their prevention practices of leptospirosis.[17] Knowledge, attitude, and practice (KAP) studies on leptospirosis conducted in Malaysia have focused mainly on describing the KAP among urban and rural communities,[18,19] residents in flood-prone areas,[20,21] town service workers,[22] wet market workers,[23] non–high-risk group,[24] and university students.[25] Yet, there are no KAP studies on leptospirosis conducted among visitors of recreational areas, which limits our ability to understand their level of awareness toward the prevention of leptospirosis. Here, we aimed to identify KAP related to leptospirosis among visitors of

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Anexo 5: Using Rhodamine B to assess the movement of small mammals in an urban slum.

DOI: 10.1111/2041-210X.13693

Check for updates

Received: 11 February 2021 | Accepted: 29 June 2021

DOI: 10.1111/2041-210X.13693

RESEARCH ARTICLE

Methods in Ecology and Evolution

Using Rhodamine B to assess the movement of small mammals in an urban slum

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Funding information
This work was supported by the CNPq-TWAS (Process No. 148306/2017-9), the Fundação de Amparo à Pesquisa do Estado da Bahia (JCB0020/2016) and the Medical Research Council (MRC) of the United Kingdom (Award No. MR/P024084/1). Open access funding provided by Swedish University of Agricultural Sciences. A.M.A. was a CNPq-TWAS Ph.D. fellow, while F.N.S. and C.G.Z. were FAPESB Ph.D. fellows at the time of this study.

Handling Editor: Robert B. O'Hara

Abstract

1. The small mammals, especially rats are pest species that are present in cities world-wide. The rat moves around and into residences and other anthropogenic structures. It is especially ubiquitous in urban slums and a threat to infrastructure and public health due to the pathogens it carries and transmits. Effective control of rat populations in most urban areas has been unsuccessful, despite several rodent control efforts. Limited information about rat movement distance has hindered identification of control units and effective scales at which to enact control during interventions.
2. We evaluated the suitability of Rhodamine B, a non-toxic biomarker, for assessing the distance travelled by rats in urban slums. We tracked rats over two campaigns between 2019 and 2020.
3. Overall, 27.9% of trapped rats showed signs of Rhodamine B in their whiskers under fluorescence microscope. This shows that our method provides a viable alternative for investigating the movement of small mammals in this area. We found that rats move up to 90 m distance in urban slums, with smaller rats travelling more actively than bigger rats.
4. Information obtained from this study should be useful in guiding efficient rodent control initiatives to reduce the risk of household rodent infestation and rodent-borne disease in urban slums.

KEYWORDS
rat, rhodamine B, rodent, slum, snap trap, zoonoses

Anexo 6: *Leptospira interrogans* biofilm formation in *Rattus norvegicus* (Norway rats) natural reservoirs

DOI: 10.1371/journal.pntd.0009736

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Leptospira interrogans biofilm formation in *Rattus norvegicus* (Norway rats) natural reservoirs

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Citation: Santos AAN, Ribeiro PS, da França GV, Souza FN, Ramos EAG, Figueira CP, et al. (2021) *Leptospira interrogans* biofilm formation in *Rattus norvegicus* (Norway rats) natural reservoirs. PLoS Negl Trop Dis 15(9): e0009736. https://doi.org/10.1371/journal.pntd.0009736

Editor: Philip Stewart, Rocky Mountain Laboratories, NIAID, NIH, UNITED STATES

Received: April 15, 2021

Accepted: August 17, 2021

Published: September 8, 2021

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: AA and PSR were fellows of National Council for Scientific and Technological Development (CNPq - https://www.gov.br/cnpq/pt-br). This study was supported by Oswaldo Cruz Foundation (FIOCRUZ - https://www.bahia.fiocruz.br/), Federal University of Bahia (UFBA - www.ufba.br), the Secretary of Health Surveillance of the State of Bahia (http://www.saude.ba.gov.br/

Abstract

Rattus norvegicus (Norway rat) is the main reservoir host of pathogenic *Leptospira*, the causative agent of leptospirosis, in urban environments. Pathogenic *Leptospira* forms biofilms in the environment, possibly contributing for bacterial survival and maintenance. Nonetheless, biofilms have not yet been studied in natural animal reservoirs presenting leptospiral renal carriage. Here, we described biofilm formation by pathogenic *Leptospira* inside the renal tubules of *R. norvegicus* naturally infected and captured in an urban slum endemic for leptospirosis. From the 65 rats carrying *Leptospira* in their kidneys, 24 (37%) presented biofilms inside the renal tubules. The intensity of leptospiral colonization in the renal tubules (OR: 1.00; 95% CI 1.05–1.1) and the type of occlusion pattern of the colonized renal tubules (OR: 3.46; 95% CI 1.20–9.98) were independently associated with the presence of *Leptospira* biofilm. Our data showed that *Leptospira interrogans* produce biofilms during renal chronic colonization in rat reservoirs, suggesting a possible role for leptospiral biofilms in the pathogenesis of leptospirosis and bacterial carriage in host reservoirs.

Author summary

Leptospirosis is an infectious disease caused by pathogenic *Leptospira* bacteria. The main reservoir hosts of *Leptospira* are the brown rats (*Rattus norvegicus*), which are chronically colonized in the kidneys. Leptospires form biofilms, which are communities of microorganisms embedded in an extracellular polysaccharidic matrix. *Leptospira* pathogenesis in reservoir hosts is poorly understood. We captured 87 brown rats from an impoverished urban community that is endemic for leptospirosis. To investigate the biofilm in the rats' kidneys, we co-localized leptospires and saccharides of the biofilm extracellular matrix in

Anexo 7: Population dynamics of synanthropic rodents after a chemical and infrastructural intervention in an urban low-income community.

DOI: 10.1038/s41598-022-14474-6

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Population dynamics of synanthropic rodents after a chemical and infrastructural intervention in an urban low-income community

Adedayo Michael Awoniyi[1,12]✉, Cristina Venegas-Vargas[2,12], Fabio Neves Souza[1,3,4], Caio Graco Zeppelini[1], Kathryn P. Hacker[5], Ticiana Carvalho-Pereira[4], Catarina Lobo Marins[4], Mayara Carvalho de Santana[3], Arsinoê Cristina Pertile[1,3], Michael Begon[6], Albert I. Ko[3,7], Peter J. Diggle[8], Mitermayer G. Reis[3,7,9], James E. Childs[7], Eduardo Mendes da Silva[1,10], Federico Costa[1,3,4,7,8] & Hussein Khalil[11]✉

Synanthropic rodents are ubiquitous in low-income communities and pose risks for human health, as they are generally resistant to control programs. However, few or no studies have evaluated the long-term effect of chemical and infrastructural interventions on rodent population dynamics, especially in urban low-income communities, or evaluated the potential recovery of their population following interventions. We conducted a longitudinal study in a low-income community in the city of Salvador (BA, Brazil) to characterize the effect of interventions (chemical and infrastructural) on the dynamics of rodent population, and documented the post-intervention recovery of their population. We evaluated the degree of rodent infestation in 117 households/sampling points over three years (2014–2017), using tracking plates, a proxy for rodent abundance/activity. We reported a significant lower rodent activity/abundance after the chemical and infrastructural interventions ($Z = -4.691$ ($p < 0.001$)), with track plate positivity decreasing to 28% from 70% after and before interventions respectively. Therefore, the combination of chemical and infrastructural interventions significantly decreased the degree of rodent infestation in the study area. In addition, no rodent population rebound was recorded until almost a year post-intervention, and the post-intervention infestation level did not attain the pre-intervention level all through the study. Moreover, among pre-treatment conditions, access to sewer rather than the availability of food was the variable most closely associated with household rodent infestation. Our study indicates that Integrated Pest Management (IPM)-approaches are more effective in reducing rodent infestation than the use of a single method. Our findings will be useful in providing guidance for long-term rodent control programs, especially in urban low-income communities.

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Anexo 8: Evaluation of the impact of chemical control on the ecology of *Rattus norvegicus* of an urban community in Salvador, Brazil.

DOI: 10.1371/journal.pone.0270568

PLOS ONE

RESEARCH ARTICLE

Evaluation of the impact of chemical control on the ecology of *Rattus norvegicus* of an urban community in Salvador, Brazil

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Citation: Pertile AC, Lustosa R, Carvalho-Pereira T, Pedra GG, Panti-May JA, Oliveira U, et al. (2022) Evaluation of the impact of chemical control on the ecology of *Rattus norvegicus* of an urban community in Salvador, Brazil. PLoS ONE 17(7): e0270568. https://doi.org/10.1371/journal.pone.0270568

Editor: Bi-Song Yue, Sichuan University, CHINA

Received: November 16, 2021

Accepted: June 13, 2022

Published: July 20, 2022

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0270568

Data Availability Statement: Data are available in Zenodo at https://doi.org/10.5281/zenodo.6672759.

Abstract

Background

The presence of synanthropic rodents, such as *Rattus norvegicus*, in urban environments generates high costs of prophylaxis and control, in large part due to the environmental transmission of the pathogenic spirochete *Leptospira interrogans*, which causes leptospirosis. In Salvador, Brazil, The Center for Control of Zoonosis (CCZ) is responsible for planning and implementing Rodent Control Programs (RCP) which are based on chemical rodenticide. However, these strategies have not been standardized for use in developing countries.

Aim

This study aimed to identify the effect of a chemical control campaign on the demographic variables of urban *R. norvegicus*, analyzing relative abundance, sex structure, body mass, and age of the population, as well as the characterization of spatial distribution among households, rodent capture campaigns and interventions.

Methods

This study was carried out during 2015 in three valleys of an urban poor community in Salvador. Individuals of *R. norvegicus* were systematically captured before (Pre-intervention) and three months (1st post-intervention) and six months (2nd post-intervention) after a chemical control intervention conducted by the CCZ in two valleys of the study area while the third valley was not included in the intervention campaign and was used as a non-intervention reference. We used analysis of variance to determine if intervention affected demographic

Anexo 9: Linking rattiness, geography and environmental degradation to spillover *Leptospira* infections in marginalized urban settings: An eco-epidemiological community-based cohort study in Brazil.

DOI: 10.7554/eLife.73120

eLife RESEARCH ARTICLE

Linking rattiness, geography and environmental degradation to spillover *Leptospira* infections in marginalised urban settings: An eco-epidemiological community-based cohort study in Brazil

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Competing interest: See page 20

Funding: See page 20

Preprinted: 22 September 2021
Received: 17 August 2021
Accepted: 14 September 2022
Published: 16 September 2022

Reviewing Editor: Niel Hens, Hasselt University, Belgium

Abstract

Background: Zoonotic spillover from animal reservoirs is responsible for a significant global public health burden, but the processes that promote spillover events are poorly understood in complex urban settings. Endemic transmission of *Leptospira*, the agent of leptospirosis, in marginalised urban communities occurs through human exposure to an environment contaminated by bacteria shed in the urine of the rat reservoir. However, it is unclear to what extent transmission is driven by variation in the distribution of rats or by the dispersal of bacteria in rainwater runoff and overflow from open sewer systems.

Methods: We conducted an eco-epidemiological study in a high-risk community in Salvador, Brazil, by prospectively following a cohort of 1401 residents to ascertain serological evidence for leptospiral infections. A concurrent rat ecology study was used to collect information on the fine-scale spatial distribution of 'rattiness', our proxy for rat abundance and exposure of interest. We developed and applied a novel geostatistical framework for joint spatial modelling of multiple indices of disease reservoir abundance and human infection risk.

Results: The estimated infection rate was 51.4 (95%CI 40.4, 64.2) infections per 1000 follow-up events. Infection risk increased with age until 30 years of age and was associated with male gender. Rattiness was positively associated with infection risk for residents across the entire study area, but this effect was stronger in higher elevation areas (OR 3.27 95% CI 1.68, 19.07) than in lower elevation areas (OR 1.14 95% CI 1.05, 1.53).

Anexo 10: Why is leptospirosis hard to avoid for the impoverished? Deconstructing leptospirosis transmission risk and the drivers of knowledge, attitudes, and practices in a disadvantaged community in Salvador, Brazil

DOI: 10.1371/journal.pgph.0000408

PLOS GLOBAL PUBLIC HEALTH

RESEARCH ARTICLE

Why is leptospirosis hard to avoid for the impoverished? Deconstructing leptospirosis transmission risk and the drivers of knowledge, attitudes, and practices in a disadvantaged community in Salvador, Brazil

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Abstract

Several studies have identified socioeconomic and environmental risk factors for infectious disease, but the relationship between these and knowledge, attitudes, and practices (KAP), and more importantly their web of effects on individual infection risk, have not previously been evaluated. We conducted a cross-sectional KAP survey in an urban disadvantaged community in Salvador, Brazil, leveraging on simultaneously collected fine-scale environmental and epidemiological data on leptospirosis transmission. Residents' knowledge influenced their attitudes which influenced their practices. However, different KAP variables were driven by different socioeconomic and environmental factors; and while improved KAP variables reduced risk, there were additional effects of socioeconomic and environmental factors on risk. For example, males and those of lower socioeconomic status were at greater risk, but once we controlled for KAP, male gender and lower socioeconomic status themselves were not direct drivers of seropositivity. Employment was linked to better knowledge and a less contaminated environment, and hence lower risk, but being employed was independently associated with a higher, not lower risk of leptospirosis transmission, suggesting travel to work as a high risk activity. Our results show how such complex webs of influence can be disentangled. They indicate that public health messaging and interventions should take into account this complexity and prioritize factors that limit exposure and support appropriate prevention practices.

OPEN ACCESS

Citation: Palma FAG, Costa F, Lustosa R, Mogaji HO, de Oliveira DS, Souza FN, et al. (2022) Why is leptospirosis hard to avoid for the impoverished? Deconstructing leptospirosis transmission risk and the drivers of knowledge, attitudes, and practices in a disadvantaged community in Salvador, Brazil. PLOS Glob Public Health 2(12): e0000408. https://doi.org/10.1371/journal.pgph.0000408

Editor: Syed Shahid Abbas, Institute of Development Studies, UNITED KINGDOM

Received: March 29, 2022

Accepted: November 7, 2022

Published: December 9, 2022

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pgph.0000408

Data Availability Statement: The datasets used and/or analyzed during the current study cannot be