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CENTRO DE PESQUISAS GONÇALO MONIZ

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Curso de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa

TESE DE DOUTORADO

AVALIAÇÃO AMBIENTAL E DO GRADIENTE SOCIAL NAS INFECÇÕES POR LEPTOSPIRA EM UMA COMUNIDADE DE SALVADOR-BAHIA

RENATO BARBOSA REIS

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Tese apresentada ao Curso de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa para obtenção do grau de Doutor**.**

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“AVALIAÇÃO AMBIENTAL E DO GRADIENTE SOCIAL NAS INFECÇÕES POR LEPTOSPIRA EM UMA COMUNIDADE DE SALVADOR-BAHIA”

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REIS, Renato Barbosa. Avaliação ambiental e do gradiente social nas infecções por leptospira em uma comunidade de Salvador-Bahia. 82 f. Tese (Doutorado) – Fundação Oswaldo Cruz, Centro de Pesquisas Gonçalo Moniz, Salvador, 2014.

RESUMO

Introdução: A leptospirose tem emergido como um importante problema de saúde pública no mundo. Esta zoonose é responsável por epidemias anuais que acometem principalmente indivíduos residentes em comunidades negligenciadas como as existentes em Salvador. Poucos estudos longitudinais foram realizados com objetivo de identificar os fatores de risco para infecção, fontes de transmissão e compreender a real dimensão da leptospirose nessas comunidades. **Objetivo:** Identificar fatores de risco para infecção prévia e novas infecções por leptospiras no contexto ambiental e socioeconômico em uma comunidade em Salvador Bahia. **Métodos:** Estudos de soroprevalência e coorte foram conduzidos na comunidade de Pau da Lima, com a realização de entrevistas domiciliares, avaliação peridomiciliar, coleta de material biológico e análises espaciais para mensurar a associação epidemiológica entre fatores de risco ambientais e as infecções. O teste de microaglutinação foi utilizado para avaliar amostras sorológicas obtidas. **Resultados:** Residir próximo aos esgotos abertos e do fundo do vale proximidade do domicílio a acúmulo de lixo e baixa renda foram associados com as infecções. A incidência anual de infecções secundárias foi de 2,3 vezes maior que as infecções primárias. **Conclusão:** O presente estudo sugere que medidas preventivas devem focalizar nas deficiências de infraestrutura que servem como fontes de transmissão. Intervenções mais efetivas precisam se concentrar especialmente nos adultos jovens e nos grupos populacionais mais desfavorecidos nestas comunidades.

Palavras-chave: Leptospirose urbana, Análise espacial, Áreas de pobreza, Fatores de risco, Análise ambiental.

REIS, Renato Barbosa. Environmental and social gradient on leptospira infection in a community assessment of Salvador-Bahia**.** 84 f. Thesis (Ph.D.) - Oswaldo Cruz Foundation, Gonçalo Moniz Research Center, Salvador, 2014**.**

ABSTRACT

Introduction: Leptospirosis has emerged as an important public health problem in the world. This zoonosis is responsible for annual epidemics affecting mainly individuals living in disadvantaged communities as existing in Salvador. Few longitudinal studies have been conducted in order to identify risk factors for infection, transmission sources and understand the real dimension of leptospirosis in these communities. **Objective:** To identify risk factors for early infection and new infections by Leptospira in environmental and socioeconomic context in a community in Salvador Bahia. **Methods:** Seroprevalence and cohort studies were conducted in Pau da Lima community, conducting home interviews peridomicilary review, collection of biological material and spatial analysis to measure the epidemiological association between environmental risk factors and infections. The agglutination test was used to evaluate serum samples obtained. **Results:** Residing next to open sewers and the valley floor near the homes of waste accumulation and low income were associated with infections. The annual incidence of secondary infections was 2.3 times higher than primary infections. **Conclusion:** This study suggests that preventive measures should focus on infrastructure deficiencies that serve as sources of transmission. More effective interventions need to focus especially on young adults and the most disadvantaged population groups in these communities.

Keywords: Urban Leptospirosis, Spatial Analysis, Areas of Poverty, Risk Factors, Environmental Analysis.

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LISTA DE ABREVIATURAS

IBGE:	Instituto Brasileiro de Geografia e Estatística
CI:	Intervalo de confiança do inglês *Confidence Interval (C.I)*
ELISA – IgM:	Reação Imunoenzimática de Captura de IgM, do inglês, *Enzyme Linked Immuno Sorbent Assay*
Epi Info:	Programa para gerenciamento e análise estatística de dados epidemiológicos
FIOCRUZ:	Fundação Oswaldo Cruz
GAM:	Modelo Aditivo Generalizado**,** do inglês, *Generalized Additive Model*
GEE Model:	Modelo de Equações de Estimação Generalizada, do inglês, *Generalized Estimation Equations Model*
GIS:	Sistema de Informação Geográfica, do inglês, *Geographic Information System*
MAT:	Teste de Aglutinação Microscópica ou Microaglutinação, do inglês, *Microagglutination Test*
No.:	Número
PCR:	Reação em cadeia de polimerase, do inglês, *Polymerase Chain Reaction*
PR:	Razão de prevalência, do inglês, *Prevalence Ratio*
R:	Programa para análises de dados estatísticos
RR:	Risco Relativo do inglês, *Risk Ratio*
OR:	Razão de chance do inglês, *Odds Ratio*

SUMÁRIO

1. INTRODUÇÃO

1.1 SOBRE A LEPTOSPIROSE

A leptospirose é uma doença, febril, causada por uma bactéria (espiroquetas patogênicas) do gênero *Leptospira* que acometem humanos e outros animais. Em seres humanos a doença se manifesta através de forma assintomática e também pode ocorrer de forma grave (BARTHI *et al*., 2003; CÉSPEDES, 2005; LEVETT, 2005). A doença é causada por uma diversidade de leptospiras patogênicas, com mais 250 sorovares descritos acomentendo o homem (LEVETT, 2001; LEVETT, 2005). É uma doença difundida no contexto global e muitas vezes confundida com outras efermidades como dengue e hepatite dentre outras (KO *et al.,* 1999; FAINE *et al.,* 1999; MCBRIDE *et al.,* 2005), por essa caracteristica torna-se uma doença muitas vezes subnotificada pelas autoridades de saúde pública (LEVETT, 2005; KO *et al.,* 1999).

A doença foi conhecida inicialmente como uma infecção ictérica, que afetava os militares em combate, trabalhadores ligados ao esgotamento sanitário, trabalhadores da mineração e agricultores de arroz. Em 1886 a descrição da doença feita por Adolf Weil que chamou a atenção para as complicações renais e hepáticas. Por este motivo, os casos humanos de leptospirose que apresentam comprometimentos renais e hepaticos são conhecidos como síndrome de Weil (WEIL, 1886).

No Japão, Ido, Inada e colaboradores (IDO *et al.,* 1915; INADA, 1916) e na Alemanha Frome e Hübener (HÜBENER; REITER, 1915; FROME, 1915) relataram o isolamento de um agente etiológico através da infecção de cobaias com tecidos de pacientes graves e concluiram os sintomas descritos eram reproduzidos nos roedores. O agente foi nomeado pelos pesquisadores japoneses como *Spirochaeta icterohaemorrhagiae* (INADA, 1916; IDO *et al.,* 1915). Após estes primeiros achados outros estudos, incluindo revisões, vêm sendo publicadas sobre a leptospirose e as leptospiras (FARR, 1995; FAINE *et al.*, 1999; VINETZ, 1996; LEVETT, 2001; CÉSPEDES, 2005; LEVETT, 2005; McBRIDE *et al.*, 2005; KO, 1999 GOARANT e PICARDEAU, 2009).

1.2 O AGENTE ETIOLÓGICO

A bactéria causadora da leptospirose é espiroqueta, helicoidal pertence ao gênero *Leptospira*, são aeróbias e sendo as: *Leptospira interrogans* as patogênicas e *Leptospira biflexa* as saprófitas de vida livre (FAINE, 1999; CÉSPEDES, 2005; HAAKE *et al.*, 2000). Mais de duzentos sorovares de leptospiras patogênicas foram descritos na literatura (FAINE, 1999; LEVETT, 2005). Os sorovares podem ter associação com as manifestações da doença, distribuição geográfica ou frequência em um determinado tipo de reservatório (VINETZ *et al*., 1996; FAINE *et al.*, 1999).

Em um estudo realizado durante um surto de leptospirose em Salvador-Bahia em 1996, foi demonstrado que em 87% dos isolados foi a *Leptospira interrogans* sorovar Copenhageni o microrganismo associado com as infecções (KO *et al.,* 1999).

1.3 OS RESERVATÓRIOS

Em ambientes rurais e urbanos (FARR, 1995; CAMPAGNOLO *et al*., 2000) diversos animais são estudados e identificados como reservatórios para a disseminação e manutenção no ambiente da bactéria causadora da leptospirose. Nos ambientes urbanizados os roedores são os principais reservatórios da bactéria e vem sendo descritos e estudados (COSTA, 2010). Os principais animais associados às infecções neste contexto são os *Rattus norvegicus* e o *Rattus rattus* (SUNBUL *et al*., 2001; LEVETT, 2005; FARIAS et al., 2008; MATHIAS *et al.*, 2008; COSTA, 2010) pelo longo período de eliminação das leptospiras no ambiente, uma vez que estes podem albergar a bactéria por até toda a vida (LEVETT, 2005; McBRIDE *et al.*, 2005). O papel epidemiológico do cão também já foi descrito como reservatório para a leptospirose humana em ambiente urbano (BROD *et al.*, 2005; BROW e PRESCOTT, 2008, FRAGA, 2008).

1.4 DIAGNÓSTICO LABORATORIAL

A leptospirose apresenta um conjunto de sintomas o que faz com que esta possa ser confundida com outras doenças, tais como dengue, hepatites virais e gripe (KO *et al*., 1999; BARTHI *et al.,* 2003), sendo necessárias análises laboratoriais específicas para auxiliar no seu diagnóstico.

Os testes disponíveis para diagnóstico laboratorial incluem Teste de Microaglutinação (MAT) e a *Enzyme Linked Immuno Sorbent Assay* (ELISA). A MAT pesquisa a aglutinação de anticorpos na adição dos diferentes sorovares como antígenos em microscopia de campo escuro. Entretanto, este teste apresenta baixa sensibilidade na fase inicial da doença além de ser de difícil manutenção nos laboratórios, porém, é um teste padrão aceito para detecção de infecções por leptospiras (FAINE *et al*., 1999). O ELISA busca a detecção de anticorpos da classe IgM, apresenta baixa sensibilidade no início do quadro clínico, mas é usado largamente em muitos países, com várias apresentações comerciais (LEVETT, 2001; SMYTHE *et al.*, 2002; CÉSPEDES, 2005; LEVETT, 2005).

Há outras técnicas disponíveis para o diagnóstico da leptospirose, como as baseadas em biologia molecular. Estas no contexto das pesquisas são relatadas como técnicas que apresentam resultados satisfatórios para sensibilidade e especificidade, porém em função dos altos custos envolvidos, ainda permanecem com baixa capacidade de disseminação na prática clínica nos países em desenvolvimento (TOYOKAWA *et al.*, 2011).

1.5 INTERAÇÕES ENTRE A DOENÇA E O AMBIENTE

A transmissão da leptospirose acontece pelo contato direto ou indireto com urina ou tecidos de animais infectados (FAINE *et al.*, 1999; LEVETT, 2005). Na vertente do risco ocupacional o contato direto é importante para compreender a origem das infecções em grupo de trabalhadores como veterinários, trabalhadores de fazendas e de abatedouros (LEVETT, 2005), além de trabalhadores que atuam na limpeza urbana de esgotos ou em contato direto com resíduos domiciliares.

O contato indireto, a partir de exposições por tempo prolongado à água contaminada, onde as leptospiras rompem a pele esteja ela integra, com pequenos ferimentos ou através da conjuntiva e adentram o organismo humano, sendo esta a forma mais frequente de contaminação, causando verdadeiras epidemias urbanas e acidentes recreacionais (BARTHI *et al*., 2003; CÉSPEDES, 2005).

Em um estudo realizado por Almeida et al. (1999) os fatores de risco para infecção por leptospira entre trabalhadores ligados às atividades de saneamento, no Rio Grande do Sul no municipio de Pelotas, evidenciaram que as infecções estavam ligadas às condições de deficiencia na infraestutura no local de residência, por possuir esgotos a céu aberto (OR=2,39; IC95% 1,01-5,64) e criar suínos (OR=7,69; IC95% 1,24-47,63) nos seus locais de residênciaforam fatores de risco identificados.

Com a realização de análises através de Sistemas de Informações Geográficas (SIG) os autores identificaram que durante uma epidemia, em Santa Cruz e Sepetiba no Rio de Janeiro, as áreas com acúmulo de lixo e com maior susceptibilidade à ocorrencia de inundações eram aquelas com maiores taxas de incidência para leptospirose (BARCELLOS e SABROZA, 2001).

Maciel et al. (2008) apontaram que características ambientais de favelas são importantes fatores para transmissão de leptospirose, aumentando em até cinco vezes o risco de aquisição de infecção se os indivíduos residirem próximo a casa de pacientes sorologicamente confirmados para esta doença.

Kawaguchi et al. (2008) descreveram a prevalência de anticorpos séricos em 23,9% (97/406) das pessoas em um estudo em comunidades rurais produtoras de arroz de uma província da República Democrática Popular do Laos, em que os fatores independentemente associados ao adoecimento por leptospirose foram coletar madeira na floresta, exposição a uma enchente na propriedade do próprio doente e ser do sexo masculino.

1.6 MAGNITUDE

1.6.1 Surtos de leptospirose

Várias condições ambientais e fatores de risco são descritos na literatura relacionados a surtos de leptospirose. Campagnolo et al. (2000) ao investigarem um surto de leptospirose em trabalhadores, alunos, veterinários e outros expostos a suínos infectados em uma fazenda da universidade no Missouri (EUA), identificaram nove casos confirmados sorologicamente (Elisa-IgM) e concluíram que lavar as mãos depois do trabalho teve um efeito protetor (OR=0,2; IC95% 0.03-0,81) à doença.

Valdés e Breijo (2001) descreveram a ocorrência de um surto, com 33 casos em pessoas ligadas a agricultura, o equivalente a 56% do total de casos ocorridos entre 1996 e 1998 no município de San Luís na província de Pina del Río (Cuba) em adultos jovens.

Sehgal et al. (2002) investigaram a ocorrência de surto de leptospirose na região de Orissa (India) ao final de dezembro de 1999 com 28 (19,7%) pessoas com evidência de infecção após um ciclone tropical, com títulos à MAT variando entre 400 a 6400, principalmente pelo sorogrupo Canicola. Bharadwaj et al. (2002) identificaram que o sorovar Copenhageni foi o responsável em 89,1% dos casos de leptospirose em um surto em área urbana de Mumbai (Índia) com 152 casos confirmados por MAT ou Elisa-IgM.

Corcho et al. (2007) relataram um surto de leptospirose durante os meses de outubro e novembro com a confirmação de 6,9% (61/885) dos casos suspeitos em diferentes cidades na província de Guantánamo em Cuba.

Góngora et al. (2008) identificaram uma prevalência, em amostra de conveniência, de anticorpos anti-leptospira em diferentes grupos com vulnerabilidade distinta, como estudantes, veterinários, magarefes, agricultores perfazendo uma prevalência desde 5,2% a 48% (trabalhadores de piscicultura) no município de Villavicencio na Colômbia.

Desai et al. (2009) descreveram um surto em coletadores de morango entre junho e setembro na Alemanha, em que o risco de adoecer aumentava em 10% (OR=1,1; IC95% 1,04-1,14) a cada dia de trabalho na chuva coletando morangos com ferimentos na pele.

Romero et al. (2010), ao realizar um corte transversal com uma amostra de conveniência, observaram uma prevalência de anticorpos anti-leptospira em 6% (51/850) dos soros de pacientes residentes em áreas urbanas de três municípios do Estado de Tolima na Colômbia.

No Brasil, Ko et al. (1999) descreveram um surto de leptospirose durante a epidemia de dengue na capital Salvador do Estado da Bahia, com 133 casos confirmados. Em 42% dos casos a leptospirose foi diagnosticada primariamente, com letalidade em 15,9%. Das amostras isoladas, 87% tratava-se de um único sorovar (*Leptospira interrogans* sorovar Copenhageni) responsável pela epidemia.

1.6.2 Prevalência de leptospirose

Em um inquérito de soroprevalência realizado com amostra randômica de 1.390 indivíduos de Salvador-Brasil, informações sobre fatores ambientais e sócio-econômicos foram coletados e o teste de microaglutinação foi utilizado para identificar infecção prévia por leptospira. A soroprevalência identificada foi 12,4%. Entre os individuos com sorologia positiva, 111/1.390 (61%) tinham títulos na microaglutinação elevados para o sorovar *Icterohaemorrhagiae*. A soroprevalência aumentou com a idade e foi similar entre homens e mulheres, houve associação entre infecção e baixo nível educacional (DIAS *et al*., 2007).

Em um estudo conduzido no municipio de Monte Negro, interior do estado de Rondônia, anticorpos contra leptospira foram indentificados em 10,2% (28/276) das pessoas que viviam em fazendas selecionadas no município em 2002. A prevalência foi maior 14,5% nos homens em relação às mulherese o contato com água de rio foi fator de risco identificado. (AGUIAR *et al.*, 2007).

Lacerda et al. (2008) descreveram em uma região de agricultura de subsistência (produção de arroz, feijão e milho) no interior do Rio Grande do Norte, a prevalência de 15,2% (44/290) de anticorpos para leptospirose na população estudada, de forma interessante, foi demonstrado que a ocorrência de leptospirose teria acontecido em períodos com baixos índices de pluviosidade.

1.6.3 Mudança do perfil de epidemiologico de transmissão da leptospirose

Nas últimas decadas é observada mudança no padrão epidemiológico da leoptospirose. Atividades esportivas, ocorridas em países desenvolvidos, são descritas na literatura associadas à ocorrência de surtosda doença. Morgan et al. (2002), relatam um surto de leptospirose ocorrido entre os participantes de um trihatlon no lago Springfiel, nos Estados Unidos em 1998. Neste estudo 11% dos 474 atletas que tiveram amostra sorologicas testadas foram positivos para leptospirose e 6% dos 248 moradores apresentou evidência sorológica de infecção. As fortes chuvas que antecederam o evento sugerem o aumento da contaminação no lago. A ingestão da água do lago foi o fator de risco identificado dentre os participantes.

Kariv et al. (2001), em um estudo durante os anos de 1985 a 1999, apontaram para a mudança do padrão da leptospirose em Israel, que antes era associada a ocupação dos pacientes com a atividades ligadas à agricultura e passou também a ser relacionada a moradores de centros urbanos.

Karande et al. (2002) detectaram um surto (n=30) em crianças residentes em área de favela de Mumbai (Índia) após a ocorrência de enchentes devido as fortes chuvas das monsões.

Nos países em desenvolvimento, a urbanização e o aumento das áreas com ocupação adensada e com deficiências de infraestrutura apontam para o possível aumento do risco de proliferação da leptospirose, e consequentemente risco de doença grave e óbitos (KO *et al*., 1999; KARIV *et al.*, 2001; KARANDE *et al.*, 2002; SARKAR *et al.*, 2002; BARTHI *et al.*, 2003; GORDILHO-SOUZA, 2008; MACIEL *et al.*, 2008; REIS *et al.*, 2008).

1.6.4 Medidas de controle contra leptospirose

As principais medidas de controle voltadas para a redução da infecção por leptospiras compreendem, a diminuição da população de animais reservatórios, implementação de barreiras para separar os reservatórios animais do habitat humano, evitar a manutenção de ambientes favoráveis para a vida dos roedores, como alimentos disponíveis próximos às casas, lixo espalhado, abrigos disponíveis e imunização de cães e gado bovino (OMS, 2003).

No Brasil, a principal medida descrita pelo Ministério da Saúde é a desratização para o controle da população de roedores (BRASIL, 2010), contudo não existem evidências de que esta medida tenha resultado na redução da incidência da leptospirose em humanos (COSTA, 2010). As medidas de controle contra a leptospirose urbana, como o controle de roedores, quando dissociadas espaço-temporalmente são falíveis frente à manutenção de áreas com deficiências na infraestrutura, como coleta de lixo irregular, esgotos abertos, principalmente nas favelas urbanas, como já demonstradas por vários autores (BARCELLOS e SABROZA, 2001; SARKAR *et al.*, 2002; DIAS *et al.*, 2007).

Estudos têm revelado que ações de saneamento básico refletem diretamente nas condições ambientais e, consequentemente, no bem estar humano, produzindo a redução na morbidade e mortalidade (FEWTRELL *et al*., 2005; RÊGO, 2002; BARRETO *et al*., 2007).

As ações de saneamento básico refletem diretamente nas condições ambientais e, consequentemente no bem estar humano, produzindo a redução na morbidade e mortalidade e estando diretamente relacionadas com a saúde da população. Essas ações além de serem fundamentalmente de saúde pública e de proteção ambiental, não deixam de ser também um bem de consumo coletivo, um serviço essencial e um direito do cidadão e um dever do Estado (BRASIL, 2004).

Os efeitos do ambiente sobre a saúde têm gerado uma grande preocupação para a sociedade na atualidade. É necessária a produção de informações que deem suporte para avaliar e sanar os problemas relativos a esta interação. As ações na área de vigilância sanitária e ambiental em saúde requerem uma compreensão ampla das questões ambientais e epidemiológicas. As evidências encontradas nos estudos apontam para a importância dos benefícios das condições adequadas de saneamento sobre alguns indicadores de saúde (FEWTRELL *et al.*, 2005). Sendo assim, a análise da situação sanitária precisa incorporar outras dimensões do estado de saúde, medida por dados de morbidade, incapacidade de acesso a serviços, qualidade da atenção, condições de vida e fatores ambientais. O uso de indicadores e análise espacial para dados sanitário-ambientais podem ser utilizados como ferramentas importantes nos estudos epidemiológicos, como forma de se estabelecer estratégias preventivas, principalmente para as populações mais expostas a riscos. (MILROY e*t al.*, 2001, DIAS *et. al.*, 2004, RÊGO, 2002).

2 JUSTIFICATIVA

A rápida urbanização e a crescente desigualdade social levaram ao aumento das áreas de aglomerado subnormal[1] no ambiente urbano, principalmente nos países em desenvolvimento. A leptospirose é uma doença negligenciada que afeta os segmentos menos favorecidos e vulneráveis da população.

A principal barreira para enfrentar esse problema de saúde pública tem sido a ausência de medidas efetivas para prevenir a leptospirose. Poucos estudos de base populacional foram realizados nas populações urbanas mais pobres visando identificar fontes de transmissão que podem ser alvo para intervenções visando à prevenção. Novos avanços metodológicos têm sido realizados nos campos de Sistemas de Informações Geográficas (SIG), mas eles não têm sido amplamente utilizados na compreensão da leptospirose e outras doenças negligenciadas (BARCELLOS e SABROZA, 2001; TASSINARI *et al*., 2008).

Neste estudo é proposta a utilização de abordagens epidemiológicas, em conjunto com uso de SIG para identificar os determinantes ambientais e sociais da transmissão da leptospirose assintomática com a realização de três estudos na cidade do Salvador. As estratégias utilizadas nestes estudos podem contribuir para aumentar o nível de conhecimento sobre os fatores de riscos e a dinâmica de contaminação de grupos de pessoas no ambiente urbano, além de servirem como modelo para estudos de outras doenças negligenciadas e problemas de saúde ambiental que afetam comunidades urbanas mais pobres no Brasil e em outros países em desenvolvimento.

[1]Definido pelo IBGE como um conjunto constituído de, no mínimo, 51 unidades habitacionais (barracos e casas) carentes, em sua maioria de serviços públicos essenciais, ocupando ou tendo ocupado, até período recente, terreno de propriedade alheia (pública ou particular) e estando dispostas, em geral, de forma desordenada e densa.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Identificar fatores de risco para infecção por leptospiras no contexto ambiental e socioeconômico em uma comunidade em Salvador Bahia.

3.2 OBJETIVOS ESPECÍFICOS

- Analisar a distribuição espacial das infecções por leptospira em uma comunidade em Salvador/BA.
- Identificar fatores de risco socioeconômicos e ambientais para novas infecções por leptospira.

4 MÉTODOS

4.1 ESCOLHA DO LOCAL DE ESTUDO

O estudo foi realizado no bairro de Pau da Lima, localizado geograficamente através das coordenadas 12º 55’ 28” Sul e 38º 26’ 07” Oeste, na cidade de Salvador, estado da Bahia. Foi definida uma área de estudo com 0,46 km², compreendendo quatro vales (Figura 01). Através de vigilância hospitalar ativa, foi identificada na área uma incidência média anual de leptospirose grave de 57,8 casos por 100.000 habitantes entre 1996 e 2001 (dados não publicados).

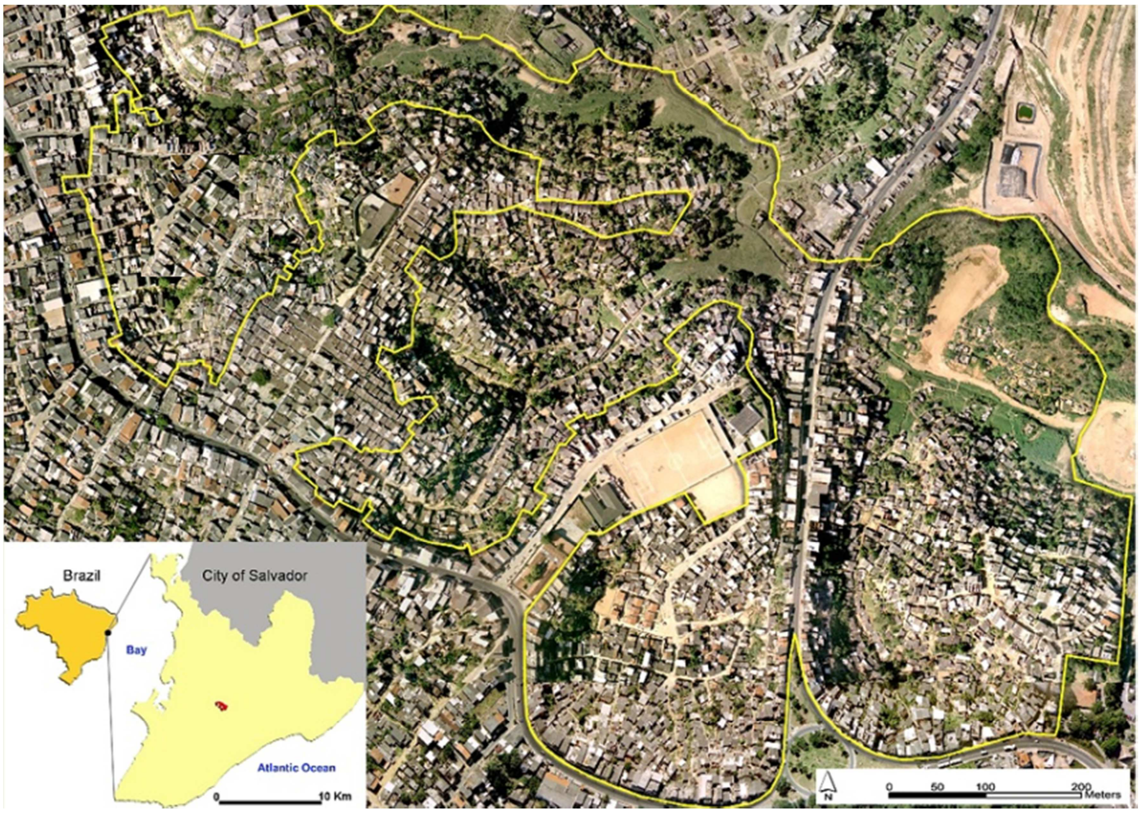

Figura 1– Delimitação da área de estudo (linha amarela) em Pau da Lima.

A equipe de estudo realizou um censo durante visitas a 3.689 domicílios, em 2003, e identificou 14.122 residentes. Foram atribuídas para as famílias numerações sequenciais vinculando-as aos domicílios e foram adicionados números decimais para identificação de cada indivíduo no domicilio. Ex.: A Casa de número 10 contendo 03 moradores, cada morador passou a identificado pela numeração 10.01 primeiro morador, 10.02 segundo morador e 10.03 terceiro morador. Foi realizada uma seleção aleatória da amostra dos domicílios a partir da base de dados contendo todos os domicílios recenseados para compor a amostra dos indivíduos e domicílios integrantes nos estudos.

4.2 CRITÉRIOS DE INCLUSÃO

Foram consideradas elegíveis para participação nos estudos as pessoas que:

- Dormissem mais que 2 noites por semana no domicilio;
- Tivessem idade maior ou igual que 5 anos e
- Fornecer consentimento para participação.

Os indivíduos foram incluídos no estudo entre abril de 2003 e maio de 2004 após fornecerem consentimento de participação através do termo de consentimento livre e esclarecido aprovado pelos comitês de ética da Fundação Oswaldo Cruz, da Comissão Nacional de Ética em Pesquisa, e *Weill Medical College of Cornell University*.

Os critérios de exclusão

- Dormir menos que duas noites por semana no domicilio;
- Ter idade inferior a 05 anos e
- Não fornecer consentimento para participação no estudo.

Após aplicar os critérios de inclusão e exclusão a população participante no estudo foi de 9.863 (69,8%) residentes.

4.3 AMOSTRAGEM DA POPULAÇÃO E INQUÉRITO DOMICILIAR

As amostragens foram realizadas através de amostragem por conglomerado, realizada por seleção aleatória simples por domicilio contendo seus respectivos moradores. Um estudo basal foi realizado entre 2003 e 2004 com 3.171 residentes (Anexo 1). No primeiro estudo, com desenho epidemiológico tipo corte transversal, foram selecionados 6.466 residentes (Figura 2).

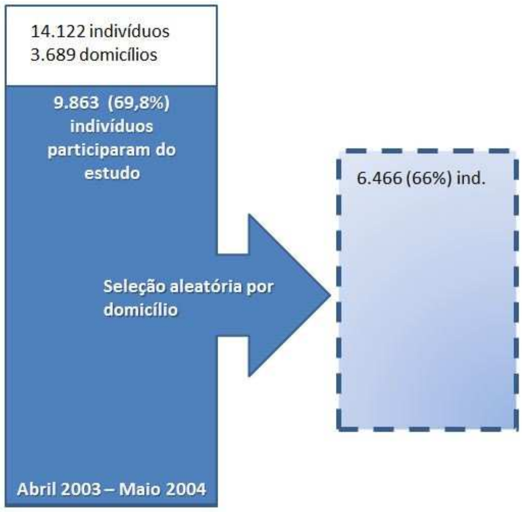

Figura 2 - Amostragem do artigo 1

Para o segundo estudo, com desenho epidemiológico longitudinal, foram selecionados 2003 indivíduos (Figura 3) dentre os 3.171 indivíduos selecionados para o estudo basal (Anexo 1).

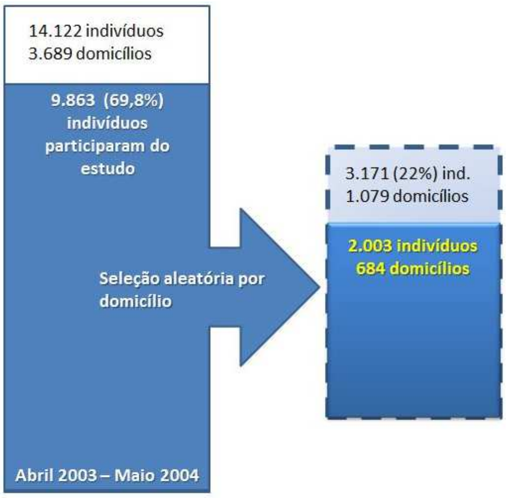

Figura 3 - Amostragem do artigo 2

A equipe de estudo era composta por agentes comunitários de saúde, enfermeiros e médicos que realizaram entrevistas durante as visitas domiciliares e aplicaram questionário padronizado para obter informações sobre os indicadores demográficos e socioeconômicos, emprego e ocupação, exposições às fontes de contaminação ambiental e potenciais reservatórios da leptospira na casa e no ambiente de trabalho. A equipe de estudo avaliou se os participantes eram alfabetizados de acordo com a capacidade destes para ler frases padronizadas e interpretar o seu significado. O trabalho informal foi definido como as atividades relacionadas com o trabalho remunerado para o qual participante não possuía a carteira profissional assinada por um empregador legal. Chefe de família foi definido como o membro que possuía o maior rendimento mensal entre os membros da família. Os participantes foram solicitados a relatar o maior número de ratos avistados nas adjacências do domicílio e local de trabalho. A equipe de estudo também questionou aos participantes sobre a criação de animais domésticos como cães, gatos e a criação de galinhas nos domicílios visando ampliar o conhecimento sobre o papel epidemiológico destes animais na transmissão da leptospirose urbana.

4.4 ANÁLISES SOROLÓGICAS

As análises sorológicas foram realizadas a partir de amostras de sangue coletadas de cada participante do estudo durante visitas domiciliares. O teste de micro aglutinação (MAT) foi realizado para avaliar a evidência sorológica de infecção prévia por Leptospira (WHO, 2003). Foi utilizado para realização dos testes um painel com cinco sorovares de referência (WHO *Collaborative*, Laboratório de Leptospirose, *Royal Tropical Institute*, Holanda) e dois isolados clínicos que contemplava *L. interrogans* sorovares Autumnalis, Canicola e Copenhageni, *L.* sorotipo *borgspetersenii* Ballum e *L.* Grippotyphosa sorotipo *kirschneri* (KO *et al.*, 1999). O uso deste painel teve o mesmo desempenho na identificação de casos confirmados por MAT de leptospirose durante a vigilância em Salvador (KO *et al.*, 1999). Nos estudos foram utilizadas diluições de 1:25, 1:50 e 1:100 para identificação da aglutinação e determinação dos títulos máximos das infecções e reinfecções. A ausência e a presença de um título positivo em anticorpos aglutinantes durante a pesquisa de base foi usada para diferenciar infecções primárias e secundárias por leptospira que ocorreram durante o acompanhamento da coorte. A infecção primária foi definida como soroconversão durante o qual o título MAT passou de negativo durante o estudo de base a um título ≥ 1:50 durante a o seguimento. A infecção secundária foi definida como um aumento de quatro vezes no título de MAT em um participante que tinha um título de ≥ 1:25 durante a pesquisa de base . A MAT foi repetida para amostras de participantes com infecções primárias e secundárias, a fim de confirmar o seu status.

4.5 DADOS ESPACIAIS

O georreferenciamento dos domicílios, ou seja, a representação destes através de feições pontuais contendo suas respectivas coordenadas foi realizado pela equipe de estudo durante o inquérito domiciliar, através de mapas impressos em escala 1: 1.500 e em etapa posterior, os mesmos foram representados graficamente na base de dados criada. A base era composta por fotografias aéreas georreferenciadas do ano de 2002 em escala 1:2000, com resolução espacial de 16 centímetros e mapas topográficos adquiridos na Companhia de Desenvolvimento Urbano do Estado da Bahia (CONDER). Foi utilizado o software ArcGIS (ESRI - *Environmental Systems Research Institute*) para criação do banco de dados geográficos. Foi construído um Modelo Digital de Terreno (MDT) tomando por base os dados topográficos (curvas de nível com equidistância de 1 metro) para a construção de superfície contendo estimativas contínuas das altitudes.

O levantamento dos dados sobre as fontes de contaminação espaciais (esgotos a céu aberto e sistema de drenagem das águas pluviais) foi realizado durante o período sazonal de fortes chuvas entre abril e agosto de 2003 e estas informações foram representadas na base através de feições lineares. Durante três levantamentos dentro deste período, a equipe de estudo mapeou os locais com acúmulo de lixo e mediram a área destes depósitos. Os locais foram representados através de feições pontuais e os valores médios para as áreas com acúmulo de lixo foram calculados e usados para as análises.

4.6 ANÁLISE DOS DADOS

As informações sobre os indivíduos e domicílios foram duplamente digitadas no software de EpiInfo versão 3.3.2 (*Centers for Diseases Controland Prevention*). Os Testes qui-quadrado e Wilcoxon foram usados para comparar dados categóricos e contínuos, respectivamente, para os indivíduos que eram elegíveis e os que não foram incluídos no estudo. O valor de $P \leq 0{,}05$ foi usado como critério para determinar significância estatística.

Análise das estimativas da densidade *Kernel* foi realizada com diferentes larguras de banda (10-120 metros) para avaliar as distribuições espaciais das densidades de participantes com evidência sorológica de anticorpos contra leptospira e a população total. O pacote estatístico R versão 2.4.1 (*R Foundation for Statistical Computing*) foi utilizado para obter estimativas de densidade ajustadas para o efeito de borda. A relação entre as densidades de *Kernel* para indivíduos com anticorpos contra leptospira e todos os indivíduos participantes da amostra foi medida para determinar a distribuição do risco espacial ajustado pela população. Foi utilizado um modelo digital do terreno de dados topográficos (software ArcGIS 3D *Analyst Extension*) para obter estimativas contínuas de altitude para a área de estudo. As distâncias, calculadas no espaço tridimensional, entre os domicílios, os esgotos abertos e áreas com acúmulo de lixo foram avaliados como *proxy* de exposição a estas fontes de contaminação ambiental. Elevação do domicílio em relação ao ponto mais baixo do vale em que se encontrava foi usado como um substituto para o risco de inundação. Modelos aditivos generalizados (GAM) foram utilizados para avaliar a forma funcional da associação entre variáveis contínuas e o risco de adquirir anticorpos contra leptospira. (HIN *et al.*,1999) bem como para geração de análise espacial com a distribuição geográfica do risco de infecção.

Regressão de Poisson (BARROS *et al*., 2003) foi utilizada para estimar o efeito de variáveis demográficas, socioeconômicas, domésticos e fatores relacionados ao ambiente de trabalho sobre a prevalência de anticorpos contra leptospira. Uma abordagem de inferência Bayesiana foi utilizada que incorporou dois efeitos aleatórios, a fim de explicar superdisperssão das mensurações em função da amostragem por conglomerados. Esta abordagem tem sido utilizada para estimar parâmetros em modelos complexos (ASHBY, 2006).

5 ARTIGO 1

Spatial modeling of leptospirosis in a urban community in Brazil

(Modelagem espacial da leptospirose em uma comunidade urbana no Brasil)

Artigo a ser revisado e submetido para publicação.

RESUMO:

A leptospirose é uma doença infecciosa de importância global que surgiu como problema de saúde urbana devido à transmissão por roedores em comunidades com sistema de saneamento básico deficientes. Este estudo teve como objetivo modelar a distribuição espacial e, ao mesmo tempo, os fatores individuais e ambientais relacionados à infecção por Leptospira em uma comunidade em Salvador-Bahia. Uma pesquisa domiciliar foi realizada com 6.466 moradores de Pau da Lima, uma comunidade na periferia de Salvador, Brasil. Avaliação sorológica foi usada para identificar indivíduos com infecção prévia. Modelos aditivos generalizados (GAM) foram utilizados para analisarco-variáveis individuais e contextuais, ao mesmo tempo estimar o risco espacial de aquisição de anticorpos contra Leptospira. A razão de chances (*Odds Ratio*) de aquisição de anticorpos contra Leptospira foi significativa para: sexo, idade, raça e variáveis socioeconômicas e fatores contextuais, como a proximidade de esgotos a céu aberto, avistar ratos e altitude. A variação espacial em risco era significativamente maior para os domicílios localizados na parte inferior dos vales. A gama de riscos espaciais diminuiu com a inclusão de co-variáveis , mas ainda mantendo o mesmo padrão. Os métodos escolhidos foram confiáveis oque nos permite identificar as áreas que devem ser priorizadas para reduzir a exposição de grupos humanosao ambiente contaminado.

Spatial modeling of leptospirosis in a urban slum area in Brazil

(Modelagem espacial da leptospirose em uma favela urbana no Brasil)

Artigo a ser revisado e submetido para publicação.

AuthorsandAffiliations

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Abstract

Leptospirosis is an infectious disease of global importance, which has emerged to be a major urban health problem due to rodent-borne transmission in urban slums. This study aimed to model the spatial distribution and, simultaneously, the individual and environmental factors related to Leptospira infection in an urban slum community in Brazil. A household survey was performed on 6,466 residents of Pau da Lima, a slum community in the periphery of Salvador, Brazil. Serological evaluation was used to identify subjects with prior infection. Generalized Additive Models were used to fit individual and contextual covariates, at the same time estimating the spatial risk of acquiring Leptospira antibodies. The odds ratio of acquiring Leptospira antibodies was significant for: sex, age, race and socioeconomic variables, and contextual factors, such as proximity of open sewers, sighting rats, animals and altitude. The spatial variation in risk was significantly larger for households located at the bottom of the valleys. The range of spatial odds ratio decreased with the inclusion of fixed covariates, but still keeping the same pattern. The methods chosen were reliable than allow us to identify areas that inhabitants actions should be focused to reduce the human contact to pathogenic Leptospira.

Keywords: Leptospirosis, spatial epidemiology, generalized additive models

Introduction

Leptospirosis is a severe infectious disease of worldwide distribution caused by a pathogenic spirochaete bacteria of the genus *Leptospira* [11]. It is able to infect a range of wild and domestic mammalian species, increasing their diffusion potential [19]. Disease transmission begins when a susceptible person has contact with water, soil, or other media contaminated with leptospires.

According to the World Health Organization [22], the incidence ranges from 0.1 to 1 cases per 100,000 population/year in temperate climates and from 10 to 100 cases per 100,000 population/year in humid tropical climates. In Brazil more than 10,000 confirmed cases were identified per year [7]. The majority of cases reported happened in large urban centers [10]. Leptospirosis cases occur throughout the year in this setting [15], indicating that endemic transmission occurs.

However, the severity of this disease varies from asymptomatic or mildly symptomatic to rapidly fatal or severe manifestations, with only a small fraction of infected individuals with pathogenic leptospira progressing to severe presentations [21]. Reliable data on its incidence and prevalence in different areas is scarce, because leptospirosis infection is generally underdiagnosed and underreported [17].

Urban epidemics are the main concern and have long been associated with poor sanitation, slum housing and flooding [15, 10]. Flooding facilitates exposure to environments contaminated with rat urine. Individual behavior, such as unprotected contact with sewer and mud cleaning, is related to higher infection rates [11], possibly due to contact with contaminated soil. Nevertheless, not much is known about the risk factors to seroconversion and to progression from mild to severe clinical manifestations. Certainly the size of the inoculum should account for differences in clinical presentation. In observational studies, however, this variables only measurable using some proxy approach, and exploring the factors associated with soil contamination, responsible potentially for the presence of viable leptospira. Although environmental and behavioral factors being universally acknowledged to relate to human leptospiral contamination [5], no previous study, to our knowledge, have examined the environmental determinants of sub-clinical infection. Uncovering the factors associated to sub-clinical leptospirosis can help to enlighten the natural history of the disease. A recent study reported that low socioeconomic status, residents of households that were located near to a refuse deposit or an open sewer, working in direct contact with sewerage or waste and flooding around the household were risk factor for acquiring urban Leptospira antibodies [13].

The aim of this study is to investigate the spatial pattern of environmental and individual factors associated with leptospirosis infections. We focused in explain possible nonlinear effect of continuous covariates such as age, domicile altitude and distance from contamination sources. The geographical coordinates of the household was the spatial component included in the models.

Methods

Study Site

The study was conducted in the Pau da Lima community which is situated in the periphery of Salvador, a city of population, 2,675,65 6inhabitants [7]in Northeast Brazil. Pau da Lima is a region comprised of four valleys in an area of0.46km², transformed into a densely-populated slum settlement due to immigration of squatters in the seventies [20, 16]. In total, 36% of the population of Salvador and28% of the population in Brazil reside in slum communities with equal or greater levels of poverty as that found in Pau da Lima [20, 16].

Study Population and data collection

A household survey was conducted in 2003, composed of interviews and questionnaires about socioeconomic status, occupation and employment and exposures to sources of environmental contamination in the household and workplace. Subjects were enrolled (n= 6,466) according to written informed consent approved by the Institutional Review Boards of the Oswaldo Cruz Foundation, Brazilian National Commission for Ethics in Research, and Weill Medical College of Cornell University.

Coordinates of each household were localized and included in a Geographic Information System (GIS). A digital terrain model of topographic data was used (ArcGIS 3D Analyst Extension software) to obtain continuous estimates of altitude for the study area and extract potential GIS predictor variables. The distances, calculated in three dimensional space, of households to nearest open drainage systems and refuse deposits were evaluated as proxies of exposure to these sources of environmental attributes. Elevation of households with respect sea level in which they were situated was used as a surrogate for flood risk.

Study Variables

The response variable leptospirosis infection (yes or no) was defined by a serovar titter greater to or equal to 1:25 to define the presence of Leptospira antibodies[13]. The variables belong to two different levels: individual and contextual (domiciliary or peri-domiciliary). Individual covariates were: sex (male or female),age, race (black or nonblack), schooling (complete or incomplete primary school education), the daily income (in US dollar), works (or worked) with sewage in the last years (yes or no) and individual contact with mud, trash or sewer (yes or no).The contextual covariates were: time living in the same household (≥15 or < 15 years),the house distance from open sewer and from trash, maximum number of rats seen in the peri-domicile (>2 or ≤ 2 rats) and presence of dogs in the peri-domicile(yes or no) and altitude of the domicile above button of valley. The geographic coordinates of the household were used to estimate the spatial effects.

Data Analysis

Crude prevalence rates and univariate odds ratio (OR) with 95% confidence intervals (CI) were reported for the categorical covariates. A univariate Generalized Additive Model (GAM) [9] were used to evaluated the functional form of the association between continuous covariates and the risk of acquiring Leptospira antibodies.

Initially, a semi parametric non-spatial model, a generalized additive model (GAM), was fitted. However, these models assume that the samples are statistically independent. As our observations are spatially related, models that ignore spatial dependence are inappropriate, as they overestimate the effect of covariates(environmental variables) and underestimate the standard errors [4]. To incorporate spatial dependence in the model we used thin plate smoothing spline logistic regression [23]. Hence, the model was of the form:

$$Y \sim Bernoulli(p)$$

$$logit(p) = \mathbf{X}\beta + s(\mathbf{Z}) + s(e, n)$$

Where $\mathbf{Y}$ is the response variable, $\mathbf{X}$is a matrix of explanatory categorical covariates and $s(\boldsymbol{Z})$is a unidimentional smooth function to estimate possible variations on the effect of the $\boldsymbol{Z}$ continuous covariates effects. The additional term, $s\ (e;\ n)$, indicate the bi-dimensional smooth function of the East and North coordinate. It models the spatial structure still present after controlling for the known risk factors. This smoothing parameter is selected by minimizing the unbiased risk estimator, which was equivalent to minimizing the expected mean square error [23].The maps present the spatial OR adjusted by all covariates included in each model. There is only one scale for the spatial effects comparison.

In multivariate analysis, all variables which had a p-value below 0.10in univariate analyses were included in the regressions. Akaike's corrected information criterions (AIC) were used to select the model [12, 6]. All statistics analysis was done in the statistical package R, version 2.7.0 [18], and with **mgcv** library [23] to fit the GAM models.

Results

The observed overall prevalence of Leptospira antibodies was 16.08% (Table 1). The prevalence in males (18.55%) is 1.32 times larger than in females. Individuals who work with sewerage or waste and had contact with mud, trash or sewer the leptospira antibodies present prevalence of 29.35% and 24.21%, respectively. Among contextual covariates the prevalence of people who live in the same household for more than 15 years is 23.96%; having seen more than two rats around the peri-domicile in recent weeks is 20.72%; dog or cat in household is approximately 19% and 21%, respectively.

Daily income and altitude above sea level of the domicile presented a linear relationship with the outcome, therefore they entered the model in the original scale. All other continuous covariates effects were non-linear (Figure 1). Age increased the odds of acquiring Leptospira antibodies until approximately twenty five years old, decreasing thereafter. The distance to the nearest open sewers and trash collection sites decreased the odds until approximated sixty and one hundred meters respectively.

Table 2 presents the results of the GAM and the spatial GAM models for three approaches: without fixed covariates, just individual covariates and both individual and contextual covariates. The last line of the table shows the AIC, always smaller for the spatial models, indicating that the place of the household is determinant to seroconversion.

The inclusion of the spatial smooth term in the models improved the fit significantly (p-value <0.001). The effects of individual and contextual covariates were similar in all models, except for race and presence of dog in the household that lost significance when the spatial effect was incorporated. Comparing the individual model, the variables with the largest effects were sex (OR 1.39, 95% IC 1.20 - 1.62) and working with sewage (OR 1.48, 95% IC 1.13 - 1.93). The effects of the contextual covariates \time of living in the same household (OR 1.40, 95% IC 1.18 - 1.65) and\the presence of rats (OR 1.34, 95% IC 1.15 - 1.55) were the largest. As for the smooth terms, the age was significant (p-value <0.001) in all models, but open sewer lost significance (p-value = 0.193) when the spatial term was included. In both full models, spatial and non-spatial, the altitude above sea level of the domicile and the daily income covariates were included as continuous and thus measured the linear protection effect for leptospirosis infection: for each meter above button of valley, the odds of acquiring Leptospira antibodies decreased 1%; for each daily dollar income increase, the odds decreased approximately 11% (full models). The best fit, according to AIC (5302.35), was the full model with individual, contextual covariates and the spatial term, which was significant (p-value <0.001) (Table 2).

In figure2 white lines depict significantly higher risk areas and black lines significantly lower risk areas considering 95% confidence intervals. The spatial pattern in all maps was similar: the region of increased risk of acquiring Leptospira antibodies was in the north, spreading towards the east. However, controlling for individual and contextual covariates the amplitude of the adjusted odds ratio decreased. The range of the odds ratio varies from 0.19 to 5.75, in the first spatial model, without fixed covariates, to 0.37 to 3.98 in the full model, indicating the explanatory power of the fixed individual and contextual covariates included.

Discussion

This work addressed two questions: the identification of potential individual and contextual factors that contribute to leptospirosis infection and the localization of high risk areas of leptospirosis contamination. The results of this work are consistent with those observed in other studies of risk factors associate with the urban leptospirosis.[1,2,3].

The larger number of women (55% of the total sample), due to absence of men from household during the field work time, could change the effect of some environmental factors even after controlling for sex covariate in the model. Another potential source of confounding that was also controlled in the models, is the time of living in the same place. Though this covariate was collected it was not validated.

There are several flexible approaches that could be used to verify the dose-response relationships and to evaluate the functional form besides the models used [8, 14]. Ourchoice however was driven by the flexibility of the model. First of all, the generalized linear approach allows any distribution to be modeled including counts or continuous variables, as long as they belong to the exponential family of probability distributions [12]. Besides the inclusion of a smooth function to deal with effects that vary according to the covariate level is a powerful tool for epidemiological understanding. Applied to spatial analysis, specifically in point pattern modeling, the bi-dimensional smooth function is an intuitive way to estimate the spatial effects and at the same time plot tolerance contours of significant high and low risk areas. The intra-domiciliary sample cluster was not a specific term in the model because the inclusion of a household random effects smooth out the more wiggly component of the spatial term, that was our main focus.

Although our study is based on the serological survey done by Reis et. al. [13], it was possible to validate their results with a larger sample. Besides, the statistical approach was able to deal with the spatial dependence and estimate, at the same time, the effects of several individual and environmental exposures and its effect on the spatial pattern. However, those covariates, either from the questionnaire or derived from the GIS were not sufficient to explain all spatial variation on the leptospirosis risk. The OR maps indicated smaller spatial e effects following the inclusion of individual and afterwards contextual covariates (Figure 2).

Despite of the overall poverty, the spatial distribution of the population in the slums is not homogeneous: the poorest people live at the lowest areas, populated by rats, where waste and flood waters accumulate. To survive in the soil, the leptospiras require neutral pH and temperature around 25°C, while salinity and pollution are inhibiting factors. The stability of the spatial pattern across models is certainly due to non- measured spatially distributed variables, suggesting the presence of unknown aspects of the ecology of this disease, including particularly the population of hosts and possible concentration areas of leptospira. However, little is known about the relationship between seroconversion and the development of severe disease and which aspects trigger the incidence of diseases clusters, in relation to environmental and socioeconomic characteristics. The dynamics of leptospirosis transmission in an urban environment is still not completely understood. Nevertheless we believe to have enough evidence to support the development of focused environmental sanitation programs to control urban rodent populations and their habitats, which in turn would reduce the contact between pathogenic Leptospira and humans.

References

[1] C. Barcellos and P. Sabrosa. The place behind the case: leptospirosis risks associated environmental conditions in a flood-related outbreak in Rio de Janeiro. Cadernos de SaudePublica, 17:1-13, 2001.

[2] C. Barcellos and P. Sabroza. Social-environmental determinants of the leptospirose outbreak of 1996 in western Rio de Janeiro: a geographical approach .international journal of environmental health research. International Journal of Environmental Health Research, 10:301-313, 2000.

[3] E. Caldas, E. Costa, and M. Sampaio. Leptospirosis in Salvador (Brazil). Clinical and laboratory aspects. Rev Inst Med Trop, 20:164-176, 1978.

[4] P. Elliott, J. Wake_eld, and N. Best. Spatial Epidemiology : Methods and Applications. 2000.

[5] S. Faine, B. Adler, C. Bolin, and P. Perolat. Leptospiraand leptospirosis, volume 2nd Edition. Melbourne, Australia, 2nd edition edition, 1999.

[6] J. Faraway. Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models, volume 66. 2005.

[7] FUNASA. Epidemiologist surveillance guide (in portuguese). Technical report, Health department, National Foundation of the Health, Brazil, Brazil, 2005.

[8] S. Gilboa, A. Correa, and C. Alverson. Use of spline regression in an analysis of maternal pre pregnancy body mass index and adverse birth outcomes: Does it tell us more than we already know ? Annals of Epidemiology, 18(3):196{205, 2007.

[9] T. Hastie and R. Tibshirani. Generalised Additive Models. 1990.

[10]A. Ko, M. Reis, C. Dourado, W. Johnson, and L. Riley. Urban epidemic of severe leptospirosis in brazil. The Lancet, 353:820 825, 1999.

[11] P. Levett. Leptospirosis. Clinical Microbiology Reviews, 13:296 326, 2001.

[12] C. Mcculloch and S. Searle. Generalized, Linear, and Mixed Models. 2000.

[13] R. Reis, G. Ribeiro, R. Felzemburgh, F. Santana, S. Mohr, A. Melendez, A. Queiroz, A. Santos, R. Ravines, W. Tassinari, M. Carvalho, M. Reis, and A. Ko. Impact of environment and social gradient on leptospira infection in urban slums. PLoS Neglected Tropical Diseases, 2(4):e228, April 2008.

[14] P. Rosenberg, H. Katki, C. Swanson, L. Brown, S. Wacholder, and R. Hoover. Quantifying epidemiologic risk factors using nonparametric regression: Model selection remains the greatest challenge. Statistics in Medicine, 22:3369{3381, 2003.

[15] U. Sarkar, S. Nascimento, R. Barbosa, R. Martins, H. Nuevo, I. Kalafanos, I. Grunstein, B. Flannery, J. Dias, L. Riley, M. Reis, and A. Ko. Populationbased case-control investigation of risk factors for leptospirosis during na urban epidemic. American Journalof Tropical Medicine andHygiene, 66:605, 2002.

[16] SECOMP. Mapamento da pobreza em areas urbanas do estado da Bahia. Cdrom, Secretaria de Combate a Pobreza e as Desigualdades Sociais, 2005.

[17] H. Silva, J. Tavares-Neto, J. Bina, and R. Meyer. Leptospirose-infecção e forma subclínica em crianças de Salvador, Bahia. Revista da Sociedade Brasileira de Medicina Tropical, 36(2), 2003.

[18] R. D. C. Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2005. ISBN 3-900051-07-0.

[19] R. H. W. Terpstra. Leptospirosis in wild animals. The Veterinary Quarterly, 18:149 150, 1996.

[20] UN-HABITAT. Slums of the world: The face of urban poverty in the new millennium.(United Nations Human Settlements Programme), Nairobi, 2003.

[21] Vado-Solís, M. Cárdenas-Marrufo, B. Jimenez-Delgadillo, A. Alzina-Lopez, H. Laviada-Molina, V. Suarez-Solís, and J. Zavala-Velazquez. Clinical epidemiological study of leptospirosis in humans and reservoirs in Yucatán, Mexico. Revista do Instituto de Medicina Tropical de São Paulo, 44(6):335{340, 2002.

[22] WHO. Human leptospirosis: guidance for diagnosis surveillance and control. Word Health Organization, Geneva, 2003.

[23] S. Wood. Generalized Additive Models: An Introduction with R. Chapman & Hall/CRC Texts in Statistical Science Series, 2006.

Figure 1 - Generalized additive models (GAM) of the association between the risk of acquiring Leptospira antibodies and continuous variables of (A) Individualage (years), (B) Distance in meters to the nearest open sewer, and (C) Distance in meters to the trash collection. The adjusted odds ratio, in the GAM model is a measure for the risk of acquiring Leptospira antibodies. Solid lines represent the point estimate; dotted lines represent upper and lower 95% confidence band.

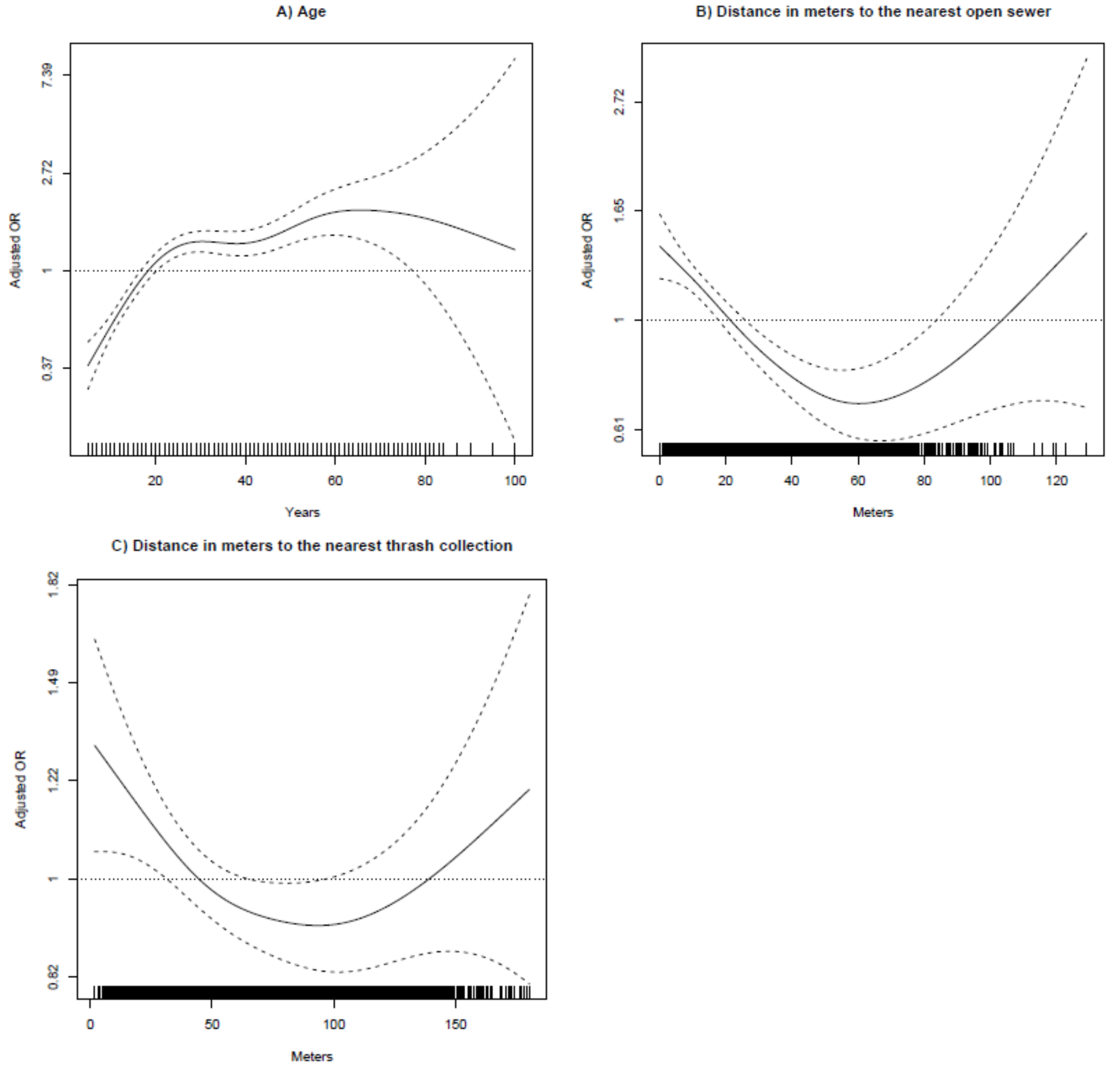
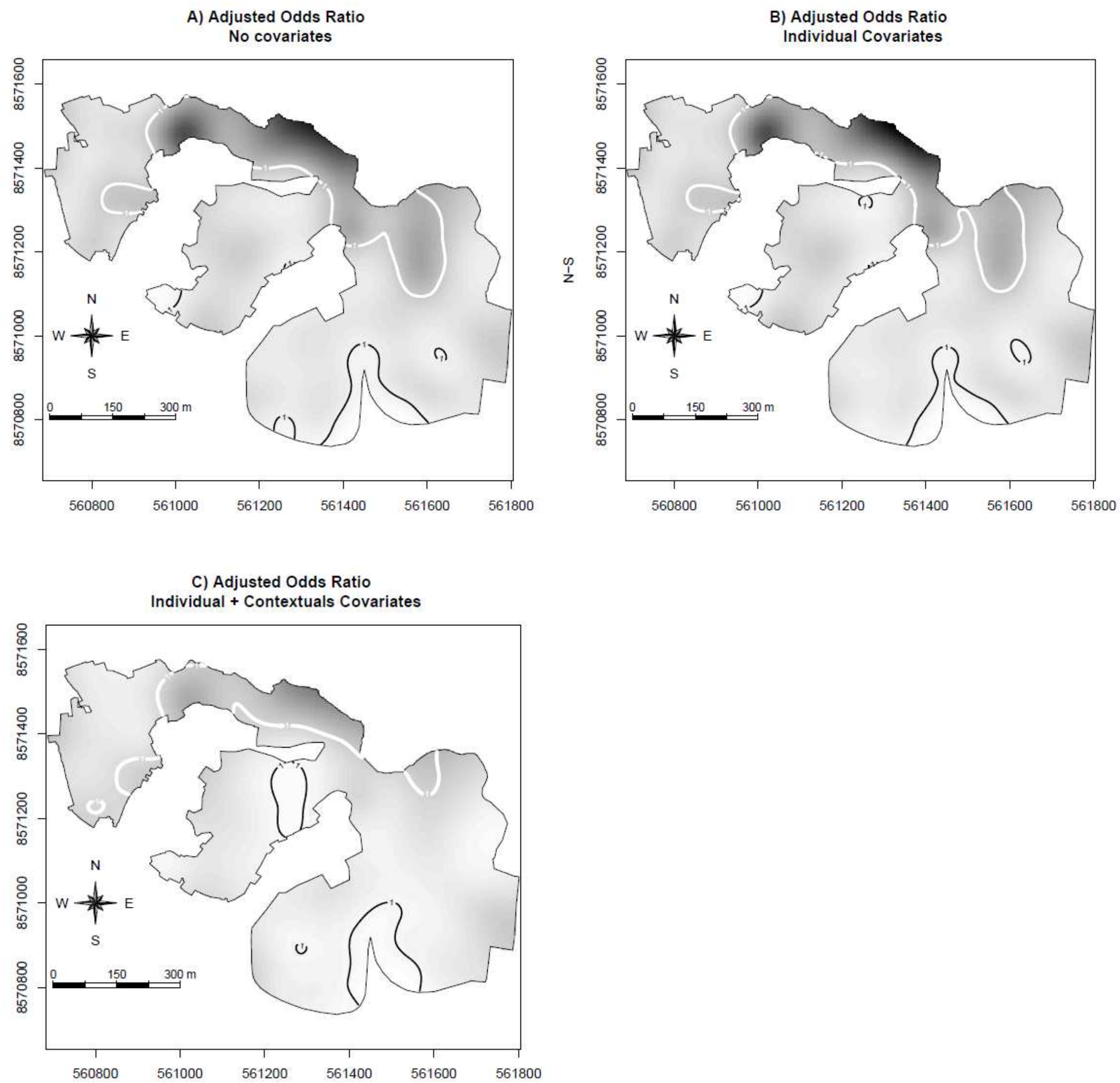


Figure 2 - Risk maps for the adjusted odds ratio of logistic spatial regressions for the of prevalence of Leptospirosis in Pau da Lima, Salvador, Bahia, Brazil,2003-2004. Black and white lines represent upper and lower 95% confidence bands, respectively. And a common legend for odds ratio surface adjusted.



Odds ratio

Table 1 - Descriptive analysis of categorical variables available for the univariate and bivariate analysis for the prevalence of Leptospirosis study in Pau da Lima, Salvador, Bahia, Brazil, 2003-2004.

	N	**Positive Results**	**Prevalence (%)**
All subjects	6,466	1,040	16.08
Individual Level			
Gender			
Males	2,927	543	18.55
Female (reference)	3,539	497	14.04
Primary school education			
Incomplete	1,536	212	16.79
Complete (reference)	4,930	828	13.80
Contact with mud, trash sewer			
yes	818	198	24.21
No (reference)	5,648	842	14.91
Race			
Black	1,916	350	18.27
Nonblack (reference)	4,550	690	15.16
Work with sewage			
Yes	368	108	29.35
No (reference)	6,098	932	15.28
Contextual level			
Living in the same household			
≥ 15 years	1,398	335	23.96
< 15 years (reference)	5,068	705	13.91
See rats near home			
> 2	2,684	556	20.72
≤ 2 (reference)	3,782	484	12.80
Dog in the household			
Yes	2,462	466	18.93
No (reference)	4,004	574	14.34

Table 2 -Adjusted Odds Ratio and others fit measurements of the logistic regression for the prevalence of Leptospirosis in Pau da Lima, Salvador, Bahia, Brazil, 2003-2004.

Logistic Models	**No Covariates models**		**Individual Models**		**Full Models**	
Variables	**Empty model OR (95% CI)**	**Spatial GAM OR (95% CI)**	**GAM OR (95% CI)**	**Spatial GAM OR (95% CI)**	**GAM OR (95% CI)**	**Spatial GAM OR (95% CI)**
Individual Variables						
Sex (male)			1.41(1.22-1.63)	1.42 (1.22-1.64)	1.40 (1.21-1.62)	1.39 (1.20-1.62)
Primary school education incomplete			1.32 (1.10-1.57)	1.25 (1.04-1.50)	1.23 (1.02-1.47)	1.22 (1.02-1.47)
Daily income (1 dollar)			0.82 (0.79-0.88)	0.86 (0.82-.091)	0.87 (0.83-0.92)	0.89 (0.84-0.93)
Contact with mud, trash and sewer (yes)			1.30 (1.07-1.58)	1.30(1.07-1.59)	1.24 (1.02-1.51)	1.26 (1.03-1.54)
Race (Black)			1.22 (1.05-1.41)	2.12 (0.96-1.30)	1.16 (1.01-1.34)	1.10 (0.94-1.28)
Work with sewer (yes)			1.53(1.18-1.98)	1.51 (1.15-1.97)	1.46 (1.12-1.90)	1.48 (1.13-1.93)
Age smooth terms (p-value)			<0.001	<0.001	<0.001	<0.001
Contextual variables						
Living in the same household					1.46 (1.24-1.72)	1.40 (1.18-1.65)
See rats (>2)					1.41 (1.22-1.62)	1.34 (1.15-1.55)
Dog in the household					1.23 (1.06-1.42)	1.16 (0.97-1.34)
Altitude above lowest point in valley (meter)					0.99 (0.98-0.99)	0.99 (0.97-0.99)
Open sewer smooth terms (p-value)					0.04	0.19
Fit measures						
Spatial smooth terms (p-value)		< 0.001		<0.001		<0.001
AIC	5882.86	5800.37	5456.28	5351.59	5360.10	5302.35

6 ARTIGO 2

Prospective study of leptospirosis transmission in an urban slum community: Role of poor environment in repeated exposures to the Leptospira agent.

[Estudo prospectivo da transmissão de leptospirose em uma comunidade de ocupação subnormal urbana: Papel do ambiente em exposições repetidas ao agente *Leptospira*].

Publicado no PLoS Neglected Tropical Diseases, em Maio de 2014.

.

RESUMO:

A leptospirose tem emergido como um problema de saúde publica nas áreas urbanas com configurações espaciais apresentando deficiências na infraestrutura de saneamento como as encontradas nas áreas de ocupação subnormal, criando as condições para a transmissão da doença. Poucos estudos prospectivos foram realizados para determinar a carga da doença, identificar fatores de risco para a infecção e fornecer informações necessárias para orientar as intervenções nessas comunidades negligenciadas. **Métodos**: Foram incluídos e seguidos nos estudo uma coorte de 2.003 residentes de uma comunidade na cidade de Salvador, Brasil. Um estudo de base e o seguimento por um ano com inquéritos sorológicos foram realizados para identificar infecções primárias e secundárias por Leptospiras, que foram definidos respectivamente como, a soroconversão e aumento de quatro vezes nos títulos no Teste de Microaglutinação (MAT) nos indivíduos com um título positivo durante o estudo de base . Foram usados modelos de regressão logística multinomial para avaliar as exposições de risco em adquirir a infecção primária e secundária. **Resultados**: Um total de 51 infecções Leptospira foram identificadas entre os 1.585 (79%) participantes que completaram o protocolo de seguimento de um ano. Maiores títulos MAT foram dirigidos contra um único *L. interrogans* sorovar Copenhageni, em 50 (98%) dos participantes infectados. Incidência anual global de infecção foi de 37,8 por 1.000 pessoas. A incidência anual de infecções secundárias era 2,3 vezes maior do que a de infecções primárias (71,4 e 31,1 infecções por 1.000 pessoas, respectivamente). Ser do sexo masculino (OR 2,88 IC 95 % 1,40-5,91) e ter menor renda familiar per capita (OR 0,54, IC 95%, 0,30-0,98 para um aumento de US $ 1 por pessoa, por dia) foram fatores de risco independentes para a infecção primária. Em contraste, o grupo de 15-34 anos de idade (OR 10,82, IC 95 % 1,38-85,08), e proximidade de residência a um esgoto a céu aberto (OR 0,95; IC 95 % 0,91-1,00 para um aumento de 1m de distância) foram fatores de risco significativos para infecções secundárias. **Conclusões**: Este estudo concluiu que os moradores de favelas tinha alto risco (> 3 % ao ano) para a aquisição de uma infecção por *Leptospira*, indicando que há grande carga da doença que é sub-reconhecida em comunidades urbanas marginalizadas. Re- infecção é evento frequente e ocorre em regiões que estão em proximidade para esgotos abertos. Para a prevenção eficaz da leptospirose, é necessário intervenções nas deficiências de infraestrutura que contribui para exposições repetidas nos habitantes desta comunidade.

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Prospective Study of Leptospirosis Transmission in an Urban Slum Community: Role of Poor Environment in Repeated Exposures to the *Leptospira* Agent

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Abstract

Background: Leptospirosis has emerged as an urban health problem as slum settlements have rapidly spread worldwide and created conditions for rat-borne transmission. Prospective studies have not been performed to determine the disease burden, identify risk factors for infection and provide information needed to guide interventions in these marginalized communities.

Methodology/Principal Findings: We enrolled and followed a cohort of 2,003 residents from a slum community in the city of Salvador, Brazil. Baseline and one-year serosurveys were performed to identify primary and secondary *Leptospira* infections, defined as respectively, seroconversion and four-fold rise in microscopic agglutination titers. We used multinomial logistic regression models to evaluate risk exposures for acquiring primary and secondary infection. A total of 51 *Leptospira* infections were identified among 1,585 (79%) participants who completed the one-year follow-up protocol. The crude infection rate was 37.8 per 1,000 person-years. The secondary infection rate was 2.3 times higher than that of primary infection rate (71.7 and 31.1 infections per 1,000 person-years, respectively). Male gender (OR 2.88; 95% CI 1.40–5.91) and lower per capita household income (OR 0.54; 95% CI, 0.30–0.98 for an increase of $1 per person per day) were independent risk factors for primary infection. In contrast, the 15–34 year age group (OR 10.82, 95% CI 1.38–85.08), and proximity of residence to an open sewer (OR 0.95; 0.91–0.99 for an increase of 1 m distance) were significant risk factors for secondary infection.

Conclusions/Significance: This study found that slum residents had high risk (>3% per year) for acquiring a *Leptospira* infection. Re-infection is a frequent event and occurs in regions of slum settlements that are in proximity to open sewers. Effective prevention of leptospirosis will therefore require interventions that address the infrastructure deficiencies that contribute to repeated exposures among slum inhabitants.

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Introduction

Leptospirosis is a bacterial disease that has emerged as a major health problem in the developing world [1]. The disease is caused by a spirochete from the genus *Leptospira*, which colonizes the kidney of a wide range of mammals [2]. Human infection occurs after direct contact with an infected animal reservoir, or water and soil contaminated with their urine [1].Infection produces a broad spectrum of clinical manifestations, which may lead from an asymptomatic and mild self-limiting febrile illness to severe disease forms with high case fatality [3]. Leptospirosis has traditionally been a sporadic rural-based disease associated with occupational risk groups such as subsistence farmers [3]. However, changes in human demography during the last 50 years have raised awareness of the emergence of leptospirosis as an urban health problem [4].

Rapid urbanization and urban poverty have led to the dramatic growth of slum settlements throughout low and middle-income countries [5]. To date one billion of the world's population reside

Author Summary

Leptospirosis is a disease that is transmitted by human contact with an environment contaminated with urine from animals, such as rodents, infected by the *Leptospira* bacteria. Human illness due to these bacteria can be mild, or can have very severe complications. Residents of urban slum settlements are at high risk for this disease, but the specific risk factors for transmission in these settlements are not understood because of the lack of prospective studies in this epidemiological setting. We performed a prospective study in a Brazilian slum community to measure the risk of infection, identify the environmental and social factors that place slum residents at risk for infection, and determine whether some individuals are at risk of repeated infections. We identified a burden of infection with leptospirosis among slum residents, and found that male gender and low income both increase the risk for infection. In addition, a significant proportion of slum residents had a second exposure to leptospirosis and re-infection occurred most frequently among young adults and the poorest members of the slum community who reside in proximity of open sewers. These risk factors are amenable to interventions aimed to reduce the burden that leptospirosis imparts in this high-risk setting.

in urban slums; this population continues to expand at rates of 10% per year [5]. As a consequence of poor sanitation in these communities, slum residents are increasingly exposed and are at risk of acquiring water and animal-borne diseases [6,7] such as leptospirosis [1,4,8]. In slum settings, endemic transmission of leptospirosis is largely due to circulation of a single serogroup, *L. interrogans* serogroup Icterohaemorrhagiae, [4,7,9,10] for which the domestic rat is the maintenance host [4,11]. In tropical urban environments, increased transmission and seasonal outbreaks occur during periods of heavy rainfall and flooding [4,12,13]. Furthermore extreme climatic events such as monsoons, typhoons and hurricanes have precipitated urban epidemics, as exemplified by the Mumbai outbreak in 2005 [14] and more recently, in the Philippines in 2009 [15] and Australia in 2011 [16].

Moreover, leptospirosis imparts a large disease burden as the cause of life-threatening infection among slum dwellers. In Brazil, more than 10,000 cases of leptospirosis are reported each year [17], the large majority of whom are residents of urban slums and require hospitalization [18] for severe complications of Weil's disease and leptospirosis-associated pulmonary hemorrhage syndrome (LPHS) [1,7,19]. Overall case fatality is >10% among reported cases from Brazil [4] and >50 70% for cases that develop LPHS [7,19]. However, severe disease represents a small fraction of the overall disease burden [20,21] and to date, prospective studies have not been performed to identify the risk of leptospirosis among slum dwellers.

Investigations of urban leptospirosis, which have used ecological [4,12,22], cross-sectional [9] and case-control study designs [23,24], have identified infrastructure deficiencies in the environment where slum dwellers reside as risk factors for acquiring leptospirosis and anti-*Leptospira* antibodies. For example, high risk of *Leptospira* transmission has been found to be associated with proximity of residence to open sewers and accumulated refuse, flood-risk areas, and areas with high rat infestation [9,12,22 26]. In addition to environmental factors, low socioeconomic status among slum residents contributes to the risk of leptospirosis [12,22] and anti-*Leptospira* antibodies [9]. However these investigations are limited by the ecological study design, or in the case of cross-sectional surveys, the use of anti-*Leptospira* antibodies, which are detected in individuals up to four or more years after infection [27,28]. To date, there are no studies that have attempted to evaluate prospectively the risk factors for leptospirosis among urban slum populations.

We previously reported the findings of a large seroprevalence survey [9] of a slum community in Salvador, a city in Northeast Brazil where 33% of the population reside in slum settlements [29] and seasonal rainfall-associated epidemics of leptospirosis occur each year [4,23]. This study found that a large proportion (15.4%) of slum inhabitants had anti-*Leptospira* antibodies, suggesting that in addition to high rates of infection, repeated exposures with the *Leptospira* agent may be occurring in this high-risk population. Herein, we report findings from a prospective investigation of this urban slum population to determine the risk of *Leptospira* infection and identify risk associations for infection.

Methods

Ethics

Participants were enrolled according to written informed consent procedures approved by the Institutional Review Boards of the Oswaldo Cruz Foundation and Brazilian National Commission for Ethics in Research, Brazilian Ministry of Health, Weill Medical College of Cornell University, and Yale School of Public Health.

Study site and participants

The cohort study was conducted in the Pau da Lima slum, a community situated in the periphery of Salvador (population, 2,675,656 inhabitants) [29], Brazil. The study site has been previously described [9]. Briefly, it comprised a four-valley area of 0.46 Km^2 with poor sanitation infrastructure. In 2003, the study team performed a census in the study site and identified 14,122 inhabitants residing in 3,689 households. The median household per capita income was US$ 1.30 per day, and most (85%) of the studied population were squatters without legal title to their domiciles. A sample of 684 (18.5%) households from a database of all enumerated households identified within the study site during the 2003 census was selected using a random number generator. Household sampling was chosen to facilitate follow-up evaluations and avoid excluding family members from a part of the study protocol in which other members are participating. The sample size of this sub-cohort was selected to detect a risk ratio of at least 2.0 for exposure risk factors, and was guided by seroprevalence surveys in this community, which identified a seroprevalence of 15% [9], and case-control investigations that determined that the frequency of risk exposures for leptospirosis is between 20 40% in community individuals [23]. All participants who slept three or more nights per week in the sampled households and had five or more years of age were eligible for enrollment in the cohort study.

Epidemiological data collection

Participants were enrolled between February 2003 and May 2004. In household visits during baseline cohort enrollment, the study team of nurse technicians, physicians and nurses administered a standardized questionnaire to obtain information on demographic and socioeconomic indicators, employment and occupation, exposures to sources of environmental contamination, and presence of potential reservoirs and domestic animals, including rats, chickens, dogs, and cats, in the household and workplace. Information on race was self-reported, and interpreted as a marker of socioeconomic status. The study team evaluated literacy according to the ability to read standardized sentences and interpret their meaning. Informal work was defined as work-related activities for which the participant did not have legal working documents. Frequent exposure to contaminated environment was defined by contact with mud, floodwater, garbage or sewage in the one-month period preceding data collection. Participants were asked to report the highest number of rats sighted within the household property and workplace site in the preceding one-month period. The head-of-household, defined as the member who earned the highest monthly income, was interviewed to determine sources and amounts of income for the household. The study team surveyed the area within <10 meters of the household to determine the presence of vegetation.

Between September and October, 2004, the study team surveyed the study site to record the location of open sewage and rainwater drainage systems. We also mapped the sites of accumulated refuse and measured the area of these deposits. Geographic Information Systems (GIS) was used to obtain three-dimensional distance from the household to the nearest drainage systems and accumulated refuse, and to the lowest point in the valley (height) [9].

Serologic evaluation

The study team collected blood samples from participants during the baseline survey and a follow-up survey conducted between October 2004 and January 2005. Sera were evaluated using the microscopic agglutination test (MAT) as previously described [9] to determine titers of agglutinating antibodies against a panel of five reference strains (WHO Collaborative Laboratory for Leptospirosis, Royal Tropical Institute, Holland) and two clinical isolates [4] The use of this reduced panel of strains, which represent five *Leptospira* serovars, Autumnalis, Canicola, Copenhageni, Ballum, and Grippotyphosa, demonstrated similar performance during laboratory confirmation of leptospirosis cases [4] and seroprevalence surveys [23,30] in studies performed in Salvador, Brazil, as the use of the WHO-recommended panel of 16 reference serovars [31]. Screening was performed with serum dilutions of 1:25, 1:50 and 1:100. When agglutination was observed at a dilution of 1:100, the sample was titrated to determine the highest agglutination titer. The absence and presence of a positive agglutinating antibody titer during the baseline survey was used to differentiate primary and secondary *Leptospira* infections which occurred during follow-up of the cohort. A primary infection was defined as seroconversion during which the MAT titer increased from negative during the baseline survey to a titer ≥1:50 during the follow-up survey. A secondary infection was defined as a four-fold rise in MAT titer in a participant who had a titer of ≥1:25 during the baseline survey. The MAT was repeated for samples of participants who were defined as having primary and secondary infections, in order to confirm their status.

Statistical methods

Epidemiological and laboratory data were double-entered using the Epi-Info for Windows software (Centers for Disease Control and Prevention, Atlanta, GA). There were no missing values for any of the analyzed variables. Data for individual participants were linked by location of residence to spatially coded information for households and environmental attributes within the study site. We used Chi-square and Wilcoxon rank sum tests to compare categorical and continuous data, respectively, between participants who were and were not selected to participate in the cohort, between participants who consented and did not consent to be enrolled in the cohort and between cohort participants who completed and who did not complete the study follow-up. A P-value of 0.05 or less in two sided testing was used as criteria for a statistically significant difference.

We calculated infection rates and 95% confidence intervals according to the Poisson distribution for primary, secondary and overall *Leptospira* infections, adjusting for the design effect of the household-based cluster sampling strategy. Only participants who completed follow up were included in the analysis. Rates were expressed as infections per 1,000 person-years of follow-up.

We applied multinomial logistic regression models in both univariate and multivariate analysis to assess the relationship between explanatory variables and the occurrence of primary and secondary infections as compared to participants without evidence for incident serological infection. Interpretation of results was based on the odds ratio and 95% confidence intervals. Confounding and interaction between independent variables were evaluated by subgroup analyses prior to performing the logistic model. The results obtained in the univariate multinomial logistic regression models were confirmed using binomial logistic regression models, for which the outcome was independently primary infection versus no infection, and secondary infection versus no infection.

Variables that had significant association at a P≤0.10 in the univariate multinomial logistic model were selected to be incorporated into a hierarchical multinomial multivariate model [32] that accounted for hierarchical inter-relationships between variables and the potential underestimation of the effects of distal determinants. The hierarchical model grouped variables into three blocks; the first block contained socioeconomic variables, such as illiteracy, educational attainment, and per capita daily household income. The second block contained household variables, such as number of residents per household, time residing at the same household, household flooding, household distances to the lowest point in valley and to nearest open sewer, presence of vegetation in <10 meters from the household, and presence of potential reservoirs in the household. The third block comprised of the individual-level variables: gender, age, contact with floodwater, sewage water or trash, excavating or cleaning an open sewer, and risk related occupations. A multinomial backward elimination strategy was then performed for each block. Variables that reached a P value ≤0.10 in each of the three blocks were then selected and grouped into a final block. Multinomial backward elimination was pursued on the final block of variables and those reaching a P value ≤0.10 associated with one of the two types of infection were included in the final model. A P value <0.05 was considered statistically significant.

Results

Recruitment and follow-up of study participants

Of the 14,122 inhabitants within the study site, 12,651 (90%) were eligible to participate in the cohort and 2,419 (19%) were randomly selected by household for study enrollment. Participants who were selected for cohort recruitment were similar to participants who were not selected in regards to median age (23 versus 24 years, respectively; P: 0.38) and proportion of males (47% versus 48%, respectively; P: 0.18). Among the 2,419 selected participants, 2,003 (83%) consented for the cohort study. Those who agreed to be a cohort member were younger than those who refused to participate (median age in years, 23 vs. 25, P: 0.02) and were less likely to be male (44% versus 61%; P: <0.001).

Of the 2,003 enrolled participants, 1,585 (79%) completed the one-year follow-up study protocol. Participants were followed for a median of 306 days (minimum of 140 and maximum of 657 days). The major reason for loss to follow-up was moving to a household

Table 1. *Leptospira* infection rates among 1,585 participants of the slum community cohort according to gender.

Type	Total			Males			Females		
	No. cases	No. PY	Rate[a] (95% CI)	No. cases	No. PY	Rate[a] (95% CI)	No. cases	No. PY	Rate[a] (95% CI)
All infections	51	1,349	37.8 (26.3–51.9)	31	569	54.5 (33.8–81.4)	20	780	25.6 (13.9–41.9)
Primary infection[b]	35	1,126	31.1 (19.9–45.4)	22	462	47.6 (26.7–76.2)	13	664	19.6 (8.9–35.6)
Secondary infection[c]	16	223	71.7 (35.8–123.6)	9	107	84.1 (31.2–170.3)	7	116	60.3 (18.7–132.8)

Abbreviations: CI: confidence intervals adjusted according to design effect, PY: person-years of follow-up.
[a]Rates expressed as infections per 1,000 person-years.
[b]Primary infection was defined as an increase in the microscopic agglutination test (MAT) titer for any of the tested serovars from zero in the first test to at least 50 in the second test.
[c]Secondary infection was defined as an increase in the MAT of four-fold from an initial titer ≥25.
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outside the study site (60% of the loss-to-follow-up participants). Participants who completed follow up differed from participants who did not in that they had a lower proportion of males (42% versus 51%; P: 0.002), had a lower educational level (23% completed primary school versus 30%; P: <0.01), and had a lower income (median household monthly income per capita US$ 39 versus 42, respectively, P: 0.03).

Leptospira infection rate

Overall, 51 (3.2%) among the 1,585 participants who completed the follow-up had serological evidence for acquisition of *Leptospira* infection. None of the participants who had *Leptospira* infection reported having been diagnosed with leptospirosis in a health care facility, hospitalized for an acute febrile illness, or identified as a case of leptospirosis during active city-wide hospital-based surveillance for leptospirosis. Highest MAT titres were observed in agglutination reactions against serovars Copenhageni and Autumnalis for samples from 50 (98%) and 1 (2%), respectively,

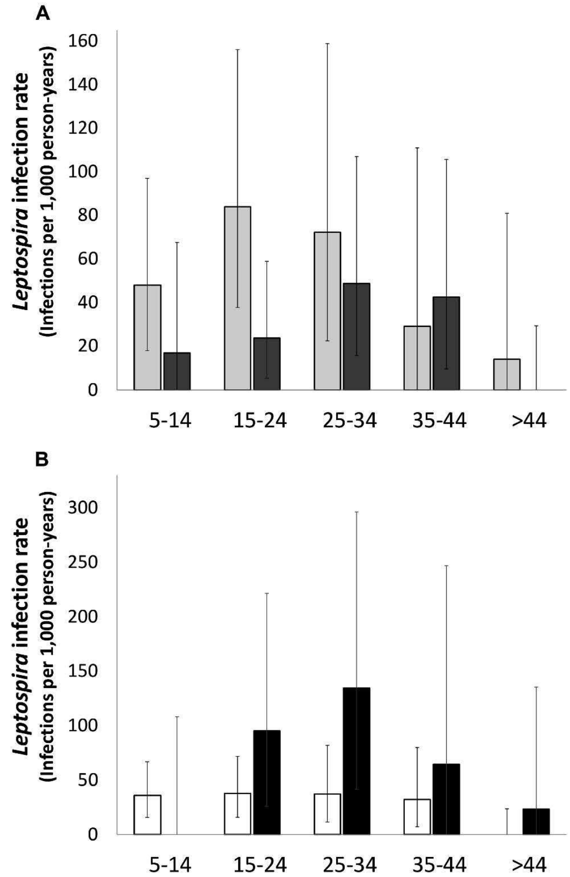

Figure 1. Age-specific attack rates for A) overall *Leptospira* infection according to gender [light grey bars, male; dark grey bars, female], and B) primary and secondary *Leptospira* infection rates [open bars, primary infection; black bars, secondary infection]. Whiskers indicate the 95% Confidence Interval for the rate, adjusted for the survey design. The x axes refer to age groups in years. Rates in y axes are shown as infections per 1,000 person-years
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of the 51 individuals with confirmed infection. The overall crude *Leptospira* infection rate was 37.8 infections per 1,000 person-years (95% CI: 26.3 51.9) (Table 1). The infection rate adjusted for age and gender of the eligible population did not significantly differ from the crude infection rates (data not shown). Infection rates were higher among the group with 15 to 24 years of age (47.9 infections per 1,000 person-years; 95% CI: 24.5 81.3) and with 25 to 34 years of age (58.1 infections per 1,000 person-years; 95% CI: 27.4 103.6). Males had 2.12 (95% CI: 1.22 3.69) times greater risk of infection than females (54.5 infections per 1,000 person-years [95% CI: 33.8 81.4] versus 25.6 infections per 1,000 person-years [95% CI: 13.9 41.9], respectively) (Table S1). The gender difference in infection risk was most prominent in the group with 15 24 years of age (RR 3.55, 95% CI: 1.28 9.88, Table S1, Figure 1A).

During the two-year period from the initiation of cohort enrollment to the end of the follow-up protocol, active hospital-based surveillance in Salvador identified five suspected cases of leptospirosis among the 12,651 inhabitants of the study site who were identified during the baseline census and were eligible to participate in the cohort study. Among suspected cases, four and one had a laboratory-confirmed and probable, respectively, diagnosis of leptospirosis. All cases had highest MAT titres directed against *L. interrogans* serovar Copenhageni. None of the leptospirosis cases was a member of the study cohort. Based on the eligible population at the site, the annual incidence for severe leptospirosis was 19.8 (7.2 43.8) cases per 100,000 population.

Primary and secondary infection rates

Of the 51 participants who had serological evidence for *Leptospira* infection, 35 had a baseline MAT titer equal to zero (1,126 person-years of follow-up) and 16 had a baseline MAT titer ≥1:25 (223 person-years of follow-up), which was defined as a marker for a previous infection. The primary and secondary infection rates were 31.1 (95% CI: 19.9 45.4) and 71.7 (95% CI: 35.8 123.6) per 1,000 person-years, respectively. The risk for secondary infections was significantly higher than primary infection during follow-up of participants who were seropositive and seronegative, respectively, at enrollment (RR: 2.31; 95% CI: 1.30 4.10).

The age groups of 15 24 and 25 34 years had the highest secondary infection rates of 95.2 and 134.6 cases per 1,000 person-years, respectively, whereas the primary infection rates in these age groups was 37.7 and 37.2 infections per 1,000 person-years, respectively ($p = 0.094$ and $p = 0.046$ respectively, Figure 1B). Although secondary infection rates were similar for males and females (RR: 1.39; 95% CI: 0.54 3.61), primary infection rates were significantly higher among males (RR: 2.43; 95% CI: 1.24 4.78) (Table S1). The gender difference in primary infection rates was greatest for the group with 15 24 years of age; males had a more than four-fold greater risk (RR: 4.22; 95% CI: 1.14 15.59) of acquiring primary infection more than females in this age group.

Risk factors for *Leptospira* infection

Due to the difference in age and gender-specific rates for primary and secondary infections, the univariate multinomial models found young adults with 15 34 years of age to have significantly increased risk for secondary infection (OR: 8.64; 95% CI: 1.12 66.65), but not for primary infection (Table 2). Male gender was associated with primary infection (OR: 2.37; 95% CI: 1.19 4.74), but not with secondary infection. Socioeconomic variables, including illiteracy, low per capita household income, and lack of a CPF card (financial identification number) were found to be risk factors for primary infection, but not for secondary infection. Each one-dollar increase in the daily per capita household income decreased the odds of primary infection by 50% (95% CI: 0.28 0.89).

In contrast to primary infection, we found that environmental attributes were significant risk factors for secondary infection in univariate analyses. Household flooding during rainy periods, proximity to open waste sewer, and three-dimensional distance of residence to the lowest point in the valley and open waste sewers had a stronger relationship with risk of secondary infection than primary infection. In contrast, risk behaviors that place participants in exposure to potentially contaminated environment near the household, such as contact with mud, sewage water, or garbage, and cleaning an open sewer, had a significant or near-significant association with both primary and secondary infections. Presence of rats at the place of residence, evaluated by the maximum number of rats seen, and sighting rats during the daytime, was not found as risk factor for primary nor secondary infection. Occupational factors, such as work that involved garbage collection and the maximum number of rats sighted at the workplace, were associated with increased risk for secondary infection in univariate analyses.

The multivariate multinomial model identified male gender (OR 2.88, 95% CI, 1.40 5.91) and per capita household income (OR 0.54 for an increase of $1 per person per day, 95% CI, 0.30 0.98) as independent risk factors for primary infection (Table 3). The model also identified age of 15 34 years (OR 10.82, 95% CI, 1.38 85.08) and proximity of the place of residence to the nearest open sewer (OR 0.95; 0.91 1.00 for an increase of 1 m distance) as risk factors for secondary infection. Contact with mud in the place of residence was found to have a non-significant association with both primary (OR 1.99, 95% CI 0.96 4.12) and secondary (OR 2.51, 95% CI 0.87 7.23) infection. Occupation-related exposures were not found to be significant risk factors for primary and secondary infection in the multivariate analyses.

Discussion

The findings of this large prospective investigation identified high rates of *Leptospira* infection among the study urban slum population, with more than 3% of the residents demonstrating serologic evidence of infection over a mean follow-up period of approximately one year. A single *L. interrogans* serogroup, serogroup Icterohaemorrhagiae, was the presumptive infecting agent, since highest agglutinating antibody titers were observed in 98% of participants with serologically-confirmed infection. Slum residents had an overall high risk for a repeat exposure and infection with the same agent. Furthermore, we identified that there are distinct risk factors for acquiring primary and secondary infection, suggesting that there exist sub-populations among slum residents who are repeatedly infected with leptospirosis.

These findings highlight the potential large and unrecognized burden of leptospirosis in urban slum settlements. There have been no comparable studies performed in slum settings that have followed large numbers of community-based participants and prospectively ascertained outcomes with standard serologic methods. A longitudinal study had been performed in Iquitos, Peru and found that annual incidence of *Leptospira* seroconversion was 288 per 1,000 persons based on IgM ELISA seroconversion during follow-up of 158 urban slum residents [33]. The much higher incidence observed in Iquitos may be due to the use of the IgM ELISA rather than the standard MAT method [33], which is more specific in detecting exposure to pathogenic leptospires [1,3]. Alternatively, the difference may reflect differences in the

Table 2. Selected univariate risk factors for *Leptospira* infection among cohort participants.

Risk factor	Uninfected n = 1,534	Primary infection n = 35		Secondary infection n = 16	
	No. or median (% or IQR)	No. or median (% or IQR)	OR (95% CI)	No. or median (% or IQR)	OR (95% CI)
Age group (years)					
5–14	466 (30.4)	13 (37.4)	1.00 (NA)	1 (6.3)	1.00 (NA)
15–34	647 (42.2)	18 (48.6)	0.94 (0.45–1.93)	12 (75.0)	8.64 (1.12–66.65)
≥35	421 (27.4)	5 (14.3)	0.43 (0.15–1.20)	3 (18.8)	3.33 (0.34–32.03)
Male gender	639 (41.7)	22 (62.9)	2.37 (1.19–4.74)	9 (56.3)	1.80 (0.67–4.86)
Illiteracy	259 (16.9)	13 (37.1)	2.80 (1.38–5.63)	5 (31.3)	2.06 (0.71–5.98)
Household characteristics					
Per capita household income (US$/day)	0.8 (0.3–1.3)	0.4 (0.1–1.0)	0.50 (0.28–0.89)	0.4 (0.1–1.0)	0.45 (0.19–1.09)
Flooding	184.0 (12.0)	4.0 (34.3)	0.95 (0.35–2.71)	5.0 (31.3)	3.35 (1.15–9.75)
Distance of residence:					
From the lowest point in valley (m)	23 (12–35)	15.0 (6.0–30.0)	0.97 (0.95–1.00)	17.5 (4.5–22.5)	0.96 (0.92–1.00)
To an open sewer (m)	19.8 (8.8–39.8)	21.6 (7.8–31.1)	1.00 (0.98–1.01)	6.9 (4.1–18.1)	0.95 (0.90–0.99)
Peridomiciliary exposures					
Cleaned clogged sewer	318 (20.7)	12 (34.3)	2.00 (0.98–4.05)	8 (50.0)	3.82 (1.42–10.27)
Contact with:					
Flood water	606 (39.6)	21 (60.0)	2.30 (1.16–4.55)	9 (56.3)	1.96 (0.73–5.30)
Sewage water	401 (26.1)	15 (42.9)	2.12 (1.08–4.18)	8 (50.0)	2.83 (1.05–7.58)
Mud	541 (35.3)	19 (54.3)	2.18 (1.11–4.27)	10 (62.5)	3.06 (1.11–8.46)
Garbage	330 (21.5)	12 (34.3)	1.90 (0.94–3.87)	7 (43.8)	2.84 (1.05–7.68)
Workplace exposures					
Refuse collection	4 (0.3)	0	0	1 (6.3)	18.56 (1.86–185.08)
Number of rats observed at workplace	3 (1.0–4.5)	5 (1.0–8.0)	1.08 (0.89–1.31)	12 (40–20.0)	1.27 (1.05–1.55)

Abbreviations: No., number; %, percentage; IQR, interquartile range; OR, odds ratio; CI, confidence interval.
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frequency of infection among slum settlements that have distinct ecological and socio-economic characteristics.

A key knowledge gap in leptospirosis centers on the natural history of the disease and specifically, the proportion of infections that progress to develop disease and severe outcomes in the setting of high endemic transmission [1,3]. Leptospirosis cases were not identified among the sample of 2,003 participants who participated in the cohort study to obtain direct estimates of the infection-to-severe-disease ratio. However, active hospital-based surveillance of the 12,651 community members that were identified during the baseline census found that the annual incidence of severe leptospirosis was 19.8 cases per 100,000 population at the study site during the cohort follow-up period. Comparison of this estimate of the severe disease incidence and the infection rate among cohort participants suggests that the infection-to-disease ratio may be as high as 191:1 (95% CI,

Table 3. Multivariate risk factors for primary and secondary infection among cohort participants.

Factor	Primary infection n = 35		Secondary infection n = 16	
	OR	95% CI	OR	95% CI
Age group (years)				
5–14	1.00		1.00	
15–34	1.17	0.54–2.56	10.82	1.38–85.08
≥35	0.64	0.22–1.90	5.39	0.54–53.59
Male gender	2.88	1.40–5.91	2.33	0.84–6.41
Per capita household income (US$/day)	0.54	0.30–0.98	0.52	0.21–1.26
Proximity to an open sewer (m)	1.00	0.99–1.02	0.95	0.91–0.99
Contact with mud near the household	1.99	0.96–4.12	2.51	0.87–7.23

Abbreviations: OR, odds ratio; CI, confidence interval.
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82 542:1). Although cohort participants with documented seroconversion did not report being hospitalized or visiting an ambulatory clinic for leptospirosis during follow-up, it is plausible that a significant proportion developed sub-clinical illness or clinical disease, which would not be identified and diagnosed as leptospirosis unless cases developed classic severe manifestations [1]. The high infection-to-disease ratio suggests that like dengue and other causes of acute fever in tropical urban environments, the burden of leptospirosis is under-recognized and significantly greater than reflected by reporting of severe cases.

The study findings indicate that the same *L. interrogans* serovar causes asymptomatic and sub-clinical infections as well as severe disease in this urban slum population. Among cohort participants with documented seroconversion, 98% had highest MAT titers that were directed against *L. interrogans* serovar Copenhageni, indicating that the Icterohaemorrhagiae serogroup was the infecting agent. We have observed that during long-term hospital-based surveillance in Salvador [4,34,35] and the study site community, >95% of the severe leptospirosis cases had highest MAT titers directed exclusively against the same serovar. *L. interrogans* serovar Copenhageni has been the sole serovar from serogroup Icterohaemorrhagiae to be isolated from this patient population during long-term surveillance [4,7], as well as rat populations from the study community [36] and the city of Salvador [10,37]. Together these findings indicate that transmission of leptospirosis is due to a single circulating serovar and provides additional evidence that *Rattus norvegicus*, the most common host of *L. interrogans* serovar Copenhageni [3,10], is the principal reservoir in this urban slum setting. Moreover, our findings raise an important question with respect to what specific factors influence disease progression and the diverse range of clinical outcomes after infection with a single serovar agent. These factors may relate to strain-specific differences within the serovar that contribute to the strain's virulence, or alternatively, host-specific susceptibility or resistance factors and types of environmental exposures that contribute to the inoculum dose during infection [1,3].

Re-infection was a frequent event among cohort participants during follow-up and raises the issue of whether natural infection confers immunity to a subsequent infection with a homologous serovar. Although there is clear evidence that immunization with live attenuated [38] and killed leptospires protects experimental animals against lethal infection [3], naturally-acquired immunity to re-infection in humans is poorly understood due to the limited number of prospective studies in well-characterized populations. A study from rural Andaman Islands used similar serologic criteria as employed in this study to prospectively identify *Leptospira* infection among school children and found that that primary infection rates were higher than secondary re-infection rates [39]. Additionally, the study observed a non-significant association between increased morbidity during follow-up and seronegative status during the baseline survey [39]. Although those findings are suggestive that a previous infection may protect against a subsequent infection, the conclusions were limited by the small numbers of clinical cases identified and potential confounding due to multiple circulating serovars.

Our study found that in an urban setting of transmission of a single serovar agent, prior exposure and infection did not confer complete protection against a subsequent serologically-ascertained, asymptomatic or sub-clinical infection with the same serovar. However, we could not determine whether prior infection protects an individual against developing clinical disease during subsequent re-infection since leptospirosis cases were not identified among cohort participants, nor could we evaluate the temporal relationship between initial infection and subsequent re-infection events due to the short follow-up period. Further prospective investigation is therefore needed to elucidate this question, which has major implications for development of an effective vaccine for leptospirosis in humans.

Urban slum residents who acquired a primary and secondary infection had some similar risk associations in common, yet also had important differences in infection rates and type of risk factors. Male gender and low socioeconomic status were independent risk factors for primary and secondary infections, although the associations with secondary infection were non-significant. Although male gender has not been identified as a risk factor for *Leptospira* infection in cohort studies performed in rural or mixed settings [33,40,41], males have a significantly higher risk for leptospirosis and anti-*Leptospira* antibodies in population-based surveillance [4,23,24] and seroprevalence surveys [9,30], respectively, in Salvador and other urban settings [42 44]. We found that for each one-dollar increase in the daily per capita household income, the odds of primary and secondary infection decreased by 46% and 48%, respectively. Poverty and low socioeconomic status may contribute to infection risk through diverse mechanisms that include psychosocial processes that promote risky behaviors and exposures with a contaminated environment, the limited use of protective clothing against abrasions that facilitate entry of the *Leptospira* spirochete [21], lack of access to amenities and social support [45] and inadequate household sanitation conditions. A previous study in the same area demonstrated that adolescents and individuals who did not complete primary school had lower levels of knowledge and practices regarding leptospirosis [46]. Our prospective study confirms the findings of a seroprevalence survey performed in the same community [9] that relatively small differences in socioeconomic level, independent of poor environment, influence the risk for leptospirosis in slum populations characterized by overall high levels of absolute poverty.

The secondary infection rate was more than twice that of the primary infection rate (RR: 2.31; 95% CI: 1.30 4.10) among cohort participants, indicating that seropositive status at the baseline survey was a marker for increased risk for infection. In contrast to primary infection, individuals who had 15 34 years of age had significantly higher risk for acquiring a secondary infection (Table 3 and Figure 1), suggesting that a sub-population of young adults are repeatedly infected with pathogenic *Leptospira*. Furthermore we found that residence in proximity of an open sewer was significantly associated with an increased risk of primary infection and not secondary infection. It seems plausible that open sewers are a risk factor for primary infection, but the magnitude of this risk association was lower than that for secondary infection and not detected in this study. Together these finding suggest that there are distinct environmental settings and behavioral factors that contribute to repeat exposures.

Seasonal flooding is a frequent occurrence in slum communities in Salvador and especially for households situated on the poorest land quality at the bottom of valleys. Ganoza and colleagues found that environmental surface water from urban slums in Peru contained high concentrations of *L. interrogans* serovar Icterohaemorrhagiae [47]. Previous cross-sectional and case-control studies found that residence in flood-prone areas and in proximity to open sewer and rainwater drainage systems were associated with increased risk for anti-*Leptospira* antibodies and leptospirosis [9,12,23,26]. The findings of this prospective study suggest that these infrastructure deficiencies of slum settlements also serve as transmission sources for repeated exposures to the *Leptospira* pathogen. Furthermore the findings demonstrate that adolescents and young adults are the primary risk group for repeated exposures and re-infection and indicate the need for specific interventions that target this high risk group.

This study has several limitations which need to be considered. The proportion of females and younger participants among study participants was greater than among non-participants. Infection rates that were adjusted for the age and gender distribution of the eligible population did not differ from crude rates, indicating that differences between enrolled and non-enrolled participants may have not introduced a significant bias in rate estimates. Among enrolled participants, 21% did not complete follow up due primarily to out-migration. This sub-group had a higher proportion of males, was better educated and had a higher income in comparison to those who completed follow up. Although we could not fully address the potential for bias, predictive factors for loss-to-follow-up were included in our modeling approach and the estimates for the risk associations may therefore be valid approximations.

In addition, our findings may not be broadly generalizable since the study was performed in a single slum community in Brazil. The incidence of *Leptospira* infection and risk associations identified in our study is expected to vary given the differences in underlying conditions of social deprivation, environmental degradation and climate where urban slum communities are situated. However the study site is a typical slum community in the city of Salvador and Brazil, where respectively, 33% [29] and 28% [5] of the population inhabit in such settlements. Furthermore, a large proportion of the one billion inhabitants of urban slums, as defined by the UN-HABITAT [5], reside in communities that have the same features of poverty, climate, poor environment and inadequate access to sanitation as found in our study site. The findings of this study may therefore be highly relevant to the situation of leptospirosis in urban slum settlements in developing countries and across tropical regions.

Furthermore, the study offers insights on the approaches needed to effectively address this neglected disease as the world's population of slum dwellers doubles to two billion by 2020 [48]. The study provides the first prospective evidence to support the assertion that defined infrastructure deficiencies in slum communities serve as transmission sources for leptospirosis. Removal of these sources, through implementation of adequate closed sewage and drainage systems, should therefore be a public health priority. Our findings also highlight the importance of adolescents and young adults as a risk group for spill-over infections. Efforts need to be made to identify and target through intervention the risky behaviors in this age group that promote recurring exposures with environmental contamination. Similarly, further work is required to identify the processes by which the social gradient of status influences unequal health outcomes within slum populations living in absolute poverty. By elucidating such mechanisms, we may not only identify effective prevention for leptospirosis, but may also identify common processes and interventions for the large range of communicable and non-communicable diseases that affect marginalized urban communities.

Supporting Information

Checklist S1 STROBE checklist.
(DOC)

Table S1 Crude *Leptospira* infection rates among 1,585 participants of the cohort, according to age and gender.
(DOCX)

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Author Contributions

Conceived and designed the experiments: MSC MGR AIK. Performed the experiments: RDMF GSR FC RBR AXTOM DF FSS SM BLdS AQS ACS RRR. Analyzed the data: RDMF GSR JEH FC WST MSC AIK. Contributed reagents/materials/analysis tools: MSC MGR AIK. Wrote the paper: RDMF GSR JEH FC AIK.

References

1. Ko AI, Goarant C, Picardeau M (2009) Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol 7: 736 747.
2. Evangelista KV, Coburn J (2010) Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol 5: 1413 1425.
3. McBride AJA, Athanazio DA, Reis MG, Ko AI (2005) Leptospirosis. Current Opinion in Infectious Diseases 18: 376 386.
4. Ko AI, Galvao Reis M, Ribeiro Dourado CM, Johnson WD, Jr., Riley LW (1999) Urban epidemic of severe leptospirosis in Brazil. Salvador Leptospirosis Study Group. Lancet 354: 820 825.
5. UN-HABITAT (2010) State of the World's Cities 2010/2011: Bridging The Urban Divide. GB. Earthscan.
6. Riley LW, Ko AI, Unger A, Reis MG (2007) Slum health: diseases of neglected populations. BMC Int Health Hum Rights 7: 2.
7. Gouveia EL, Metcalfe J, de Carvalho AL, Aires TS, Villasboas-Bisneto JC, et al. (2008) Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. Emerg Infect Dis 14: 505 508.
8. Lau CL, Smythe LD, Craig SB, Weinstein P (2010) Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Transactions of the Royal Society of Tropical Medicine and Hygiene 104: 631 638.
9. Reis RB, Ribeiro GS, Felzemburgh RDM, Santana FS, Mohr S, et al. (2008) Impact of Environment and Social Gradient on Leptospira Infection in Urban Slums. PLoS Neglected Tropical Diseases 2: e228.
10. de Faria MT, Calderwood MS, Athanazio DA, McBride AJA, Hartskeerl RA, et al. (2008) Carriage of Leptospira interrogans among domestic rats from a high endemic urban setting for leptospirosis in Brazil. Acta Trop 108: 1 5.
11. Pereira MM, Matsuo MGS, Bauab AR, Vasconcelos SA, Moraes ZM, et al. (2000) A clonal subpopulation of Leptospira interrogans sensu stricto is the major cause of leptospirosis outbreaks in Brazil. Journal of Clinical Microbiology 38: 450 452.
12. Barcellos C (2000) Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: a geographical approach. International Journal of Environmental Health Research 10: 301 313.
13. Kupek E, de Sousa Santos Faversani MC, de Souza Philippi JM (2000) The relationship between rainfall and human leptospirosis in Florianopolis, Brazil, 1991 1996. Braz J Infect Dis 4: 131 134.
14. Maskey M, Shastri JS, Saraswathi K, Surpam R, Vaidya N (2006) Leptospirosis in Mumbai: post-deluge outbreak 2005. Indian J Med Microbiol 24: 337 338.
15. Amilasan AS, Ujiie M, Suzuki M, Salva E, Belo MC, et al. (2012) Outbreak of leptospirosis after flood, the Philippines, 2009. Emerg Infect Dis 18: 91 94.
16. Smith JK, Young MM, Wilson KL, Craig SB (2012) Leptospirosis following a major flood in Central Queensland, Australia. Epidemiol Infect 141: 585 590.
17. Health Surveillance Secretary BMoH (2007) [Leptospirosis case notification records, Brazil].
18. Souza V, Brant JL, Arsky ML, Araújo WN (2010) Evaluation of the Brazilian National Epidemiological Surveillance System for Leptospirosis - Brazil, 2007. Cad Saude Col 18: 115 117.

19. Marotto PC, Nascimento CM, Eluf-Neto J, Marotto MS, Andrade L, et al. (1999) Acute lung injury in leptospirosis: clinical and laboratory features, outcome, and factors associated with mortality. Clin Infect Dis 29: 1561 1563.
20. Ashford DA, Kaiser RM, Spiegel RA, Perkins BA, Weyant RS, et al. (2000) Asymptomatic infection and risk factors for leptospirosis in Nicaragua. American Journal of Tropical Medicine and Hygiene 63: 249 254.
21. Phraisuwan P, Whitney EA, Tharmaphornpilas P, Guharat S, Thongkamsamut S, et al. (2002) Leptospirosis: skin wounds and control strategies, Thailand, 1999. Emerg Infect Dis 8: 1455 1459.
22. Oliveira DS, Guimaraes MJ, Portugal JL, Medeiros Z (2009) The socio-demographic, environmental and reservoir factors associated with leptospirosis in an urban area of north-eastern Brazil. Ann Trop Med Parasitol 103: 149 157.
23. Sarkar U, Nascimento SF, Barbosa R, Martins R, Nuevo H, et al. (2002) Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. American Journal of Tropical Medicine and Hygiene 66: 605 610.
24. Maciel EAP, de Carvalho ALF, Nascimento SF, de Matos RB, Gouveia EL, et al. (2008) Household Transmission of Leptospira Infection in Urban Slum Communities. PLoS Neglected Tropical Diseases 2: e154.
25. Segura ER, Ganoza CA, Campos K, Ricaldi JN, Torres S, et al. (2005) Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. Clin Infect Dis 40: 343 351.
26. Barcellos C, Sabroza PC (2001) The place behind the case: leptospirosis risks and associated environmental conditions in a flood-related outbreak in Rio de Janeiro. Cad Saude Publica 17 Suppl: 59 67.
27. Lupidi R, Cinco M, Balanzin D, Delprete E, Varaldo PE (1991) Serological follow-up of patients involved in a localized outbreak of leptospirosis. J Clin Microbiol 29: 805 809.
28. Cumberland P, Everard CO, Wheeler JG, Levett PN (2001) Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979 1989. Eur J Epidemiol 17: 601 608.
29. IBGE (2010) Subnormal Agglomerates - First Results. In: Estatística IBdGe, editor. Rio de Janeiro. pp. 1 259.
30. Dias JP, Teixeira MG, Costa MC, Mendes CM, Guimaraes P, et al. (2007) Factors associated with Leptospira sp infection in a large urban center in northeastern Brazil. Rev Soc Bras Med Trop 40: 499 504.
31. Goris MG, Leeflang MM, Loden M, Wagenaar JF, Klatser PR, et al. (2013) Prospective evaluation of three rapid diagnostic tests for diagnosis of human leptospirosis. PLoS Negl Trop Dis 7: e2290.
32. Victora CG, Huttly SR, Fuchs SC, Olinto MT (1997) The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. Int J Epidemiol 26: 224 227.
33. Johnson MA, Smith H, Joeph P, Gilman RH, Bautista CT, et al. (2004) Environmental exposure and leptospirosis, Peru. Emerg Infect Dis 10: 1016 1022.
34. Barocchi MA, Ko AI, Ramos Ferrer S, Tucunduva Faria M, Galvao Reis M, et al. (2001) Identification of new repetitive element in Leptospira interrogans serovar copenhageni and its application to PCR-based differentiation of Leptospira serogroups. Journal of Clinical Microbiology 39: 191 195.
35. Gouveia EL, Metcalfe JMD, de Carvalho ALF, Aires TSF, Villalobos-Bisneto JC, et al. (2008) Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. Emerg Infect Dis 14: [serial on the Internet]. Available: http://wwwnc.cdc.gov/eid/article/14/3/07-1064_article.htm.
36. Costa F, Porter F, Rodrigues G, Farias H, de Faria MT, et al. (2014) Infections by Leptospira interrogans, Seoul Virus, and Bartonella spp. Among Norway Rats (Rattus norvegicus) from the Urban Slum Environment in Brazil. Vector Borne Zoonotic Dis 14: 33 40.
37. Caldas EM, Costa E, Sampaio MB (1978) Leptospirosis in the city of Salvador (Brazil). Some aspects clinics and of laboratory. Rev Inst Med Trop S Paulo 20: 90 106.
38. Adler B, de la Pena Moctezuma A (2010) Leptospira and leptospirosis. Vet Microbiol 140: 287 296.
39. Vijayachari P, Sugunan AP, Murhekar MV, Sharma S, Sehgal SC (2004) Leptospirosis among schoolchildren of the Andaman & Nicobar Islands, India: low levels of morbidity and mortality among pre-exposed children during an epidemic. Epidemiol Infect 132: 1115 1120.
40. Everard CO, Maude GH, Hayes RJ (1990) Leptospiral infection: a household serosurvey in urban and rural communities in Barbados and Trinidad. Annals of Tropical Medicine and Parasitology 84: 255 266.
41. Vijayachari AP, Sugunan AP, Murhekab MV, Sharma S, Sehgal SC (2004) Leptospirosis among schoolchildren of the Andaman & Nicobar Islands, India: low levels of morbidity and mortality among pre-exposed children during an epidemic. Epidemiol Infect 132: 1115 1120.
42. Jansen A, Stark K, Schneider T, Schoneberg I (2007) Sex differences in clinical leptospirosis in Germany: 1997 2005. Clin Infect Dis 44: e69 72.
43. Childs JE, Schwartz BS, Ksiazek TG, Graham RR, LeDuc JW, et al. (1992) Risk factors associated with antibodies to leptospires in inner-city residents of Baltimore: a protective role for cats. Am J Public Health 82: 597 599.
44. Daher E, Zanetta DM, Cavalcante MB, Abdulkader RC (1999) Risk factors for death and changing patterns in leptospirosis acute renal failure. Am J Trop Med Hyg 61: 630 634.
45. Marmot M (2001) Inequalities in health. N Engl J Med 345: 134 136.
46. Navegantes de Araujo W, Finkmoore B, Ribeiro GS, Reis RB, Felzemburgh RD, et al. (2013) Knowledge, attitudes, and practices related to Leptospirosis among urban slum residents in Brazil. Am J Trop Med Hyg 88: 359 363.
47. Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, et al. (2006) Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic Leptospira. PLoS Medicine 3: 1329 1340.
48. United Nations Human Settlements Programme (2003) The challenge of the slums: Global report on human settlements; London and Sterling: Earthscan Publications Ltd. p., editor.

7 DISCUSSÃO GERAL

A leptospirose em Salvador ocorre de forma endêmica, com surtos epidêmicos sazonais e estes são identificados nas mesmas comunidades a cada ano. Estas comunidades têm como características as deficiências na infraestrutura de saneamento que servem como fontes de transmissão da leptospirose no contexto urbano (CALDAS *et al.*, 1979; KO *et al.*, 1999).

O primeiro trabalho realizado nesta população é um estudo com desenho epidemiológico tipo corte transversal (Anexo 1). Foram selecionados randomicamente 1.079 domicílios contendo a população de 3.171 participantes. A prevalência identificada foi de 15,4%, valor este semelhante à detectada por um estudo realizado na cidade em 1998 que encontrou uma prevalência de 12,4% da população (DIAS *et al.*, 2007). Mais de 89% das infecções foram associadas ao sorovar Copenhageni o que aponta ao elo epidemiológico para o rato como principal reservatório para a *Leptospira* no contexto urbano, (KO *et al.*, 1999; PEREIRA *et al.*, 2000; BARROCHI *et al.*, 2001; ROMERO *et al.*, 2006).

As análises epidemiológicas demonstram as associações com fatores demográficos como idade e sexo, sendo os indivíduos do sexo masculino e do grupo etário com idade igual ou superior a 45 anos apresentando maior associação com infecção.

Os fatores associados às configurações espaciais e à pobreza relacionados à infecção prévia por Leptospira foram: as deficiências em saneamento, identificadas com o auxílio de Sistema de Informação Geográfica e modelagem estatística, tais como, áreas com risco para alagamento com esgotos abertos, proximidade do domicílio a acúmulo de lixo, ver ratos (*proxy* para infestação de roedores no ambiente), fatores esses já identificados nesta e em outras regiões geográficas demonstrados em estudos sobre a transmissão da leptospirose (KO et al., 1999; BARCELLOS e SABROZA, 2000; SARKAR *et al.*, 2002; GANOZA *et al.*, 2006; MACIEL *et al.*, 2008).

As análises espaciais através da razão de *Kernel* demonstram que domicílios dos indivíduos positivos agrupavam-se áreas de invasão recentes no fundo dos vales.

A criação de galinha não tem um elo com plausibilidade biológica na transmissão da leptospirose descrita na literatura. Criar galinhas no peridomicílio se manteve estatisticamente significante neste estudo, o que possibilita hipotetizar que o fator que está influenciando esta associação, relaciona-se com as condições improvisadas para a criação das galinhas, oferecendo abrigo, comida e água para os roedores, fazendo com que estes estejam presentes nas regiões adjacentes aos domicílios, contribuindo desta forma, para a transmissão do patógeno aos humanos.

O fator sócio-econômico relacionado com a baixa renda esteve associado de forma independente à infecção por *Leptospira*. Cada acréscimo de 1 dólar na renda familiar per capita reduziu em 11% o risco de infecção (IC 95%, 5%-18%), apontando para a existência de um gradiente social que estaria influenciando o risco de infecção nesta comunidade.

Este estudo de soro prevalência apresenta algumas limitações:

A primeira é inerente ao desenho do estudo, pois se trata de um estudo de corte transversal, não havendo a possibilidade de estabelecer relação de causalidade entre exposição e desfecho. Os efeitos relacionados com esta limitação serão minimizados com a realização de um estudo de coorte nesta comunidade.

A segunda limitação está relacionada com inclusão da população no estudo, pois, o grupo dos indivíduos que não participaram era composto em sua maioria por jovens do sexo masculino, entretanto, quando comparados ao grupo dos participantes, as taxas de prevalência ajustadas por idade e sexo não apresentaram diferenças das não ajustadas.

A terceira limitação diz respeito às infecções identificadas que estão sujeitas a terem ocorridas antes do período de análise, pois é descrito na literatura que os anticorpos podem persistir por longo período (MCBRIDE *et al*, 2005).

O Primeiro artigo apresentado nesta tese abordou duas questões: a identificação de potenciais fatores individuais e contextuais que contribuem para a infecção por leptospirose e a localização de áreas de alto risco de contaminação por esse agente. Os resultados deste trabalho são consistentes com os observados em outros estudos de fatores de risco associados com a leptospirose urbana (BARCELLOS e SABROZA, 2001; BARCELLOS *et al.,* 2003).

A abordagem linear generalizada permite a realização de modelagem a qualquer distribuição, incluindo contagens ou variáveis contínuas, desde que eles pertencem à família exponencial de distribuições de probabilidade. A inclusão de uma função de suavização para lidar com os efeitos que variam de acordo com o nível da co-variável é uma poderosa ferramenta para a compreensão epidemiológica do risco. Aplicado à análise espacial, especificamente, a função de suavização bidimensional é uma ferramenta para estimar os efeitos espaciais e, ao mesmo tempo os limites de tolerância de áreas com alto e baixo risco significativo. O feito do desenho com a coleta de dados por conglomerado (por domicilio) forma o cluster da amostra intra- domiciliares não foi um termo específico no modelo.

Embora este estudo seja baseado no inquérito sorológico descrito no artigo 1, foi possível validar os resultados com uma amostra maior. Além disso, a abordagem estatística foi capaz de lidar com a dependência espacial e estimativa, ao mesmo tempo, os efeitos de várias exposições individuais e ambientais e ao seu efeito sobre o padrão espacial. No entanto, essas co-variáveis , a partir dos dados coletados com as entrevistas ou derivados do SIG não eram suficientes para explicar toda a variação espacial no risco de adquirir leptospirose. Como demonstrado nos mapas contendo menores efeitos espaciais após a inclusão de co-variáveis individuais e contextuais (Figura 2 - Artigo 1).

Apesar da pobreza em geral, a distribuição espacial da população nas favelas não é homogêneo: as pessoas mais pobres vivem nas áreas mais baixas, infestada por ratos, onde há uma maior possibilidade de ocorrer inundações. No entanto, pouco se sabe sobre a relação entre a soro conversão e o desenvolvimento de doença grave e quais os aspectos que estão relacionados às características ambientais e socioeconômicas. A dinâmica da transmissão da leptospirose em um ambiente urbano ainda não é completamente compreendida. Entretanto, acreditamos ter elementos suficientes para apoiar o desenvolvimento de programas de saneamento ambiental voltados para controlar as populações de roedores urbanos e seus habitats, que por sua vez reduziria o contato entre Leptospira patogênica e os seres humanos.

Algumas limitações são encontradas neste estudo: A primeira aponta para a necessidade de que os modelos usados devam ser validados em outras situações epidemiológicas. A segunda limitação está relacionada com a maior participação de mulheres (55% do total da amostra mesmo selecionada aleatoriamente) que pode alterar o efeito de alguns fatores ambientais, mesmo após o controle de co-variável sexo no modelo total.

O segundo artigo vem fornecer elementos para auxiliar a compreensão do fenômeno de transmissão da leptospirose urbana através de um estudo de coorte. Os resultados obtidos após o seguimento de um ano indicam altas taxas de infecção por *Leptospira*, com mais de 3% dos participantes apresentando evidência sorológica de infecção. A *L. interrogans* sorogrupo *Icterohaemorrhagiae* foi o sorovar infectante com títulos mais elevados observados em >95 % dos participantes com infecção confirmada sorologicamente. Os participantes com evidência sorológica para infecção prévia por leptospira na avaliação basal apresentaram elevação em mais de quatro vezes o risco de adquirir uma nova infecção, em comparação com os participantes que não tinham evidência de infecção prévia no início do estudo. Além disso, os resultados sugerem que há fatores de risco distintos para aquisição da infecção primária e secundária, o que nos leva a hipotetizar que existem subpopulações entre os moradores das comunidades negligenciadas que são repetidamente infectados por leptospira.

Os resultados deste estudo destacam a carga elevada da leptospirose em comunidades negligenciadas (KO, *et al.*, 2009). Entre os poucos estudos prospectivos realizados para estudar a leptospirose (JOHNSON *et al.*, 2004), não há estudos comparativos que acompanharam um número elevado de participantes. Um estudo de 158 moradores de uma comunidade urbana em Iquitos, Peru descobriu que a incidência anual de leptospira (soroconversão) foi 288 por 1.000 pessoas (JOHNSON *et al.*, 2004). A maior incidência observada em Iquitos pode ser devido à utilização do método para diagnóstico (ELISA de IgM), em vez de teste de microaglutinação (JOHNSON *et al.*, 2004), que pode ser mais específico para a detecção de soroconversões associadas com exposições à leptospiras patogénicas (KO, *et al.*, 2009; MCBRIDE *et al.*, 2005). As distintas incidências podem refletir diferenças na frequência de infecção entre as áreas que apresentam características ambientais e socioeconômicas diferenciadas.

A lacuna principal de conhecimento sobre a história natural da leptospirose é especificamente, a proporção de infecções que evoluem para desenvolver desfechos graves (KO, *et al.*, 2009; MCBRIDE *et al.*, 2005). Com base na vigilância hospitalar de membros da comunidade identificados durante o estudo basal, verificou-se que a incidência anual de leptospirose grave foi de 19,8 casos por 100.000 habitantes durante o período do estudo. Não foi identificado um número suficiente de eventos entre o sub- grupo de participantes da coorte para obter estimativas precisas sobre a relação de infecção a grave da doença. Eventos de soro conversão podem ter sido associados a infecções assintomáticas, mas é possível que uma proporção tenha desenvolvido doença sub- clínica não sendo assim, detectados por vigilância hospitalar (ASHFORD *et al.*, 2000). A alta taxa de infecção sugere que, assim como a dengue e outras causas de febre aguda em ambientes urbanos tropicais, o ônus da leptospirose é subreconhecido e significativamente maior do que refletida pela notificação de casos graves.

Os títulos de MAT indicam que o mesmo sorotipo parece causar infecções subclínicas, bem como doença grave em Salvador. Soro conversões entre 96 % dos participantes eram dirigidos contra *L. interrogans* sorovar *Copenhageni*. Este sorotipo também foi associado com os cinco casos de leptospirose hospitalizados identificadas no nosso censo, >95% das formas graves da doença em Salvador (KO *et al.*, 1999; BAROCCHI *et al.*, 2001; GOUVEIA *et al.,* 2008). *Rattus norvegicus* é o reservatório mais comum do sorovar *Copenhageni* nesta comunidade (COSTA, 2010) e de outras comunidades urbanas (LEVETT, 2001, BHARTI *et al*., 2003; KO *et al.*, 1999; PEREIRA *et al.*, 2000). Portanto, intervenções orientadas para controlar a população de ratos podem ter efeitos para reduzir a transmissão Leptospira e a doença em comunidades urbanas.

Os dados sugerem que reinfecções nesta comunidade é um evento frequente, pois, a incidência de infecção secundária por Leptospira foi 2,3 vezes maior do que a incidência de infecção primária. Pouco se conhece sobre o risco de reinfecção da leptospirose e se existe imunidade adquirida naturalmente. Esta descoberta também sugere que a infecção assintomática com leptospiras patogênicas não confere imunidade protetora de uma reinfecção subsequente, conforme definido por critérios sorológicos, no cenário epidemiológico de um único sorotipo que circula Leptospira patogênica.

Homens de baixa renda apresentaram maior risco de infecção primária. Este achado foi consistente com estudos de soroprevalência e de surtos anteriores em Salvador (KO, et al, 1999) . No entanto, o sexo masculino não foi associado com a infecção em estudos de coorte anteriores (JOHNSON, *et al.*, 2004; EVERARD, *et al.*, 1990, VIJAYACHARI, *et al.*, 2004). Cada incremento de um dólar no rendimento per capita domiciliar diário diminuiu o risco de infecção secundária em 46%. A pobreza pode determinar o risco de infecção por diversos mecanismos, incluindo comportamentos de risco, como o uso limitado de equipamentos de proteção individual tornando o indivíduo mais susceptível à aquisição de infecção, pois o patógeno penetra mais facilmente em pele lesionada (PHRAISUWAN, *et al.*, 2002), acesso deficitário às instalações e as condições sanitárias inadequadas de família (MARMOT, 2001). Além disso, os indivíduos que relataram contato com a lama tinha uma associação com as taxas de infecção mais elevadas. Os resultados deste estudo corroboram os achados anteriores (estudo basal – Anexo 1) que indicam a existência do gradiente social que afeta a transmissão da leptospirose independentemente do ambiente.

As infecções secundárias apresentaram diferentes fatores de risco independentes, quando comparadas com as infecções primárias. As infecções recorrentes foram mais comuns a ocorrer entre os adultos com idade 15-34 anos e entre os indivíduos que viviam perto de um esgoto a céu aberto, indicando que há um grupo de moradores da comunidade com alto risco de serem repetidamente infectados com Leptospira. Diferentemente da infecção primária, o sexo masculino e ter baixa renda não foram fatores de risco independentes para a infecção secundária, entretanto, o poder de detectar essas associações pode ter sido limitado pelo tamanho da amostra na investigação longitudinal.

Fontes ambientais de contaminação (esgotos a céu aberto e lama) são fatores específicos para a infecção por Leptospira nesta comunidade. Inundação sazonal é comum em áreas com ocupação subnormal em ambiente urbano e esses eventos são relatados com maior frequência nos domicílios situados nas áreas mais pobres nas proximidades do fundo dos vales e dos esgotos abertos.

Este estudo apresenta algumas limitações que merecem considerações. A proporção de mulheres mais jovens é maior entre as participantes do estudo do que entre os não-participantes. No entanto, as taxas de infecção primária e secundária ajustados para a distribuição etária e sexo da população elegível não diferiram entre taxas brutas, o que sugere que as diferenças entre os participantes inscritos e não- inscritos não podem ter introduzido um viés significativo nas estimativas.

Entre os participantes inscritos, 21% não completaram o acompanhamento devido, principalmente à emigração. Entre aqueles que não completaram acompanhamento tiveram há maior proporção de homens, assim como, de indivíduos mais instruídos e com uma renda maior, em comparação com aqueles que completaram acompanhamento. Apesar da perda de seguimento, as estimativas são representativas da população mais negligenciada que vive continuamente na comunidade por longos períodos.

O uso de sorologia para identificar a ocorrência de infecção também pode ter introduzido viés da taxa de infecção por Leptospira, ou seja, a infecção pode ter ocorrido em qualquer momento entre o início do estudo e o acompanhamento da avaliação sorológica, uma vez que não foi possível determinar a data exata da infecção.

O estudo descreve uma alta taxa de infecção por leptospira, por meio de infecções recorrentes que afeta as subpopulações mais pobres e negligenciadas desta comunidade urbana. Além disso, confirmou em um estudo prospectivo que a falta de infraestrutura de saneamento básico é o fundamental determinante para leptospirose. Portanto, abordar os fatores sociais e ecológicos que influenciam a aquisição de infecção por leptospira deve ser uma prioridade para prevenir leptospirose em áreas urbanas com ocupação subnormal.

8 CONCLUSÃO

Os resultados apresentados nesta tese sugerem que:

- Estratégias relacionadas com a prevenção da disseminação da leptospirose em ambiente urbano devem ser possíveis através da universalização do acesso ao saneamento básico e de estratégias para identificar e modificar os determinantes sociais que causam aumento no risco de infecção.
- Os fatores de risco encontrados neste estudo não incluíram variáveis ocupacionais, este estudo aponta para o ambiente peridomiciliar como local onde há maior probabilidade de ocorrer à transmissão.
- Reinfecção pelo mesmo sorotipo Leptospira ocorre a uma taxa elevada e identificar fatores de risco específicos para infecção recorrente na população de estudo avaliada.
- Estes resultados têm implicações para as medidas de controle potenciais destinadas a reduzir a leptospirose entre a população urbana mais vulnerável a partir localização geográfica do risco de infecção.

9 REFERÊNCIAS

ABDULKADER, R. C. R. M.; SILVA, M. V. The kidney in leptospirosis. **Pediatr. Nephrol.**, v. 23, p. 2111–2120, 2008.

ABGUEGUEN, P. et al. Clinical aspects and prognostic factors of leptospirosis in adults. Retrospective study in France. **J. Infect.,** v. 57, p. 171-178, 2008.

AGAMBODI, S.B. et al. A preliminary study on prevalent serovars of leptospira among patients admitted to teaching Hospital, Kandy, Sri Lanka. **Indian J. Med. Microbiol.**, v.26, n.4, p.405-406, 2008.

AGUDELO-FLORES, P.; RESTREPO-JARAMILLO, B.N.; ARBOLEDA-NARANJO, M. Situación de la leptospirosis en el Urabá antioqueño colombiano: estudio seroepidemiológico y factores de riesgo en población general urbana. **Cad. Saúde Públ.,** v. 23, n. 9, p. 2094-2102, 2007.

AGUIAR, D. M. etal.Anti-*Leptospira* spp. and anti-*Brucella* spp. antibodies in humans from rural area of Monte Negro municipality, state of Rondônia, Brazilian western amazon. **Braz. J. Microbiol.,** v. 38, p. 93-96, 2007.

ALMEIDA,L.P.; MARTINS, L.F.S.; BROD, C.S. Fatores de risco associados à presença de anticorpos antileptospira em trabalhadores do serviço de saneamento ambiental. **Ciência Rural**, v. 29, n. 3, p. 511-516, 1999.

ANDREADIS, E. A.; AGALIOTIS, G. D.; MOUSOULIS, G.P. Leptospirosis presenting in a woman with fulminant hepatic failure from Wilson's disease: a case report. **J. Med. Case Rep.,** v. 4, p. 256-258, 2010.

ARSKY, M.L.S. et al. Probable areas of infection and ambience of occurrence of human leptospirosis in Brazil (2001 – 2003). **Rev. Cubana Med. Trop**., v. 57, n. 1, p. 59-60, 2005.

ASHBY, D. Bayesian statistics in medicine: A 25 year review. **Stat. Med**., p. 3589–3631, 2006.

ASKLING, H.H. et al. Serologic analysis of returned travelers with fever, Sweden .**Emerg. Infect. Dis**., v. 15, p. 1805-1808, 2009.

BARANTON, G. P. D. Trends in leptospirosis epidemiology in France. Sixty-six years of passive serological surveillance from 1920 to 2003. **Int. J. Infect. Dis**., v. 10, p. 162-170, 2006.

BARCELLOS, C. et al. Distribuição espacial da leptospirose no Rio Grande do Sul, Brasil: recuperando a ecologia dos estudos ecológicos. **Cad. Saúde Públ.**, v. 19, n. 5, p.1283-1292, 2003.

BARCELLOS, C.; SABROZA, P.C. The place behind the case: leptospirosis risks and associated environmental conditions in a flood-related outbreak in Rio de Janeiro. **Cad. Saúde Públ.** v. 17 (Supl.), p. 59-67, 2001.

BARROS, A. J.; HIRAKATA, V. N. Alternatives for logistic regression in crosssectional studies: An empirical comparison of models that directly estimate the prevalence ratio. **BMC Med. Res. Methodol.,** v. 3, p. 21, 2003.

.

BASACA-SEVILLA, V. et al. An outbreak of leptospirosis in an agricultural penal colonyin the Philippines. **Phil. J. Microbiol. Infect. Dis.**,v. 10, n. 2, p. 73-82, 1981.

BHARADWAJ, R. et al. An urban outbreak of leptospirosis in Mumbai, Índia. **Jpn. J. Infect. Dis.**, v. 55, p. 194-196, 2002.

BHARTI, A.R. et al. Leptospirosis: a zoonotic disease of global importance. **Lancet Infec. Dis.**, v. 3, n. 12, p. 757-771, 2003.

BOURHY, P. et al. Isolation and characterization of new *Leptospira* genotypes from patients in Mayotte (Indian Ocean). **Plos Neglected Trop. Dis.**, v. 4, n. 6, p. e724, 2010.

BRANDÃO, A. P. et al. Macroscopic agglutination test for rapid diagnosis of human leptospirosis. **J. Clin. Microb.**, v. 36, n. 11, p. 3138-3142, 1998.

BRASIL. Leptospirose. In: **Guia de bolso – Doenças Infecciosas e Parasitárias**. 5ed. Brasília: Ministério da Saúde, 2010. p.274-282.

BROCKMANN, S. et al. Outbreak of leptospirosis among triathlon participants in Germany, 2006. **BMC Infect. Dis.**, v. 10, p. 91, 2010.

BROD, C. S. et al. Evidência do cão como reservatório da leptospirose humana: isolamento de um sorovar, caracterização moleculare utilização em inquérito sorológico. **Rev. Soc. Bras. Med. Trop.**, v. 38, n. 4, p. 294-300, 2005.

BROW, K.; PRESCOTT, J. Leptospirosis in the family dog: a public health perspective. **CMAJ**, v. 178, n. 4, p. 399–401, 2008.

BRUCE, M.G. et al. Leptospirosis among patients presenting with dengue-like illness in Puerto Rico. **Acta Trop.,** v. 96, p. 36-46, 2005.

BRUM,L.; KUPEK, E.Record linkage and capture-recapture estimates for underreporting of human leptospirosis in a Brazilian health district. **Braz. J. Infect. Dis.,** v. 9, n. 6, p.515-520, 2005.

CAMPAGNOLO, E.R. et al. Analysis of the 1998 outbreak of leptospirosis in Missouri in humans exposed to infected swine. **JAVMA**, v. 216, n. 5, p. 676-682, 2000.

CÉSPEDES, M.Z. Leptospirosis: Enfermedad zoonótica reemergente. **Rev. Peru Med. Exp. Salud Publ.**, v. 22, n. 4, p. 290-307, 2005.

CHIDAMBARAM, N. et al. Leptospirosis: clinical presentation and sorovar correlation. **J. Comm. Dis.**, v. 39, n. 2, p. 105-108, 2007.

CHIERAKUL, W. et al. Activation of the coagulation cascade in patients with leptospirosis, **Clin. Infect. Dis.,** v. 46, p. 254-260, 2008.

CHOU, Y.L.; CHEN,C.S.; LIU, C.C. Leptospirosis in Taiwan, 2001–2006. **Emerg. Infect. Dis**., v. 14, n. 5, p. 856-857, 2008.

CICERONI, L. et al. Epidemiological trend of human leptospirosis in Italy between 1994 and 1996. **Eur. J. Epidemiol.,** v. 16, p. 79-86, 2000.

CLAVEL, M. et al. Leptospirosis: An unusual cause of ARDS. **Crit. Care Res. Pract.,** p. 1-3, 2010.

CLERKE, A.M. et al. Clinical profile of leptospirosis in South Gurajat. **J. Postgraduate Med**., v. 48, n. 2, p.117-118, 2002.

CODEÇO, C. T. et al. A stochastic model for ecological systems with strong nonlinear response to environmental drivers: application to two water-borne diseases. **J. R. Soc. Interface**, v. 5, p. 247–252, 2008.

CORCHO, D. B. et al. Brote de leptospirosis humana en la provincia Guantánamo. **Rev. Cubana Med. Trop.**, v. 59, n. 1, p.24-29, 2007.

COSTA, E. et al. Formas graves de leptospirose: aspectos clínicos, demográficos e ambientais. **Rev. Soc. Bras. Med. Trop.**, v. 34, n. 3, p. 261-267, 2001.

COSTA, F.**Estudos ecológicos sobre reservatórios urbanos de leptospirose em Salvador**. 127 f. il. Tese (Doutorado). Salvador: Fundação Oswaldo Cruz, Instituto de Pesquisas Gonçalo Moniz, 2010.

COSTA, G.F.; ANDRADE, V.A.; BROCK, D.R. Características clínicas, laboratoriais e epidemiológicas de pacientes com suspeita de febre hemorrágica em um centro de referência em doenças infecciosas. **Rev. Bras. Med**., v. 65, n. 9, p. 292-296, 2008.

CRODA. J. et al. Leptospira Immunoglobulin-like proteins as a serodiagnostic marker for acute leptospirosis. **J. Clin. Microbiol**., v. 45, n. 5, p. 1528-1534, 2007.

CUMBERLAND, P.; EVERERARD, C.O.R.; LEVETT, P.N. Assessment of the efficacy of an IgM-Elisa and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. **Am. J. Trop. Med. Hyg**., v. 61, n. 5, p. 731-734, 1999.

DE FARIA, M.T. et al. Carriage of Leptospira interrogans among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. **Acta Trop.,** v. 108, n. 1, p. 1-5, 2008.

DE MASI, E., VILAÇA, P., RAZZOLINI, M. T. Environmental conditions and rodents infestation in Campo Limpo district, São Paulo municipality, Brazil. **Int. J. Environ. Health Res**., v. 19, n. 1, p.1-16, 2009

DESAI, S. et al. Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. **Clin. Infect. Dis**., v. 48, n. 6, p. 691-697, 2009

DIAS, J. P. et al. Factors associated with Leptospira sp infection in a large urban center in northeastern Brazil. **Rev. Soc. Bras. Med. Trop**., v. 40, n. 5, p. 499-504, 2007.

ELLIS,T. et al. Under recognition of leptospirosis during a dengue fever outbreak in Hawaii, 2001–2002.**Vector-borne and Zoonotic Diseases**, v. 8, n. 4, p. 541-547, 2008.

EVERARD, J.D. LEPTOSPIROSIS. In: Cox, F.E.G. **The Wellcome Trust Illustrated History of Tropical Diseases.** London: The Wellcome Trust, 1996. p.111-119.

FAINE, S. et al. **Leptospira and leptospirosis**. 2[a.] ed. Melbourne, Australia: Medisci, 1999.

FARIAS, M.T. et al. Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. **Acta Trop**., v. 108, n. 1, p. 1-5, 2008.

FARR, R. Leptospirosis. **Clin. Infec. Dis.**, v. 21 ,n. 1, p1-6, 1995.

FRAGA, D. B. M. **Avaliação do papel do cão como reservatório na transmissão da leptospirose urbana**. 127 f. il. Tese (Doutorado). Salvador: Fundação Oswaldo Cruz, Instituto de Pesquisas Gonçalo Moniz, 2008.

GALLOWAY, R. L. et al. Assessing cost effectiveness of empirical and prophylactic therapy for managing leptospirosis outbreaks. **Epidemiol. Infect.,** v. 137, n. 9, p. 1323-1332, 2009.

GÓNGORA, A. et al. Seroprevalence of *Leptospira sp* in population groups of Villavicencio, Colombia. **Rev. Salud Publ.,** v. 10, n. 2, p. 269-278, 2008.

GONSALEZ, C.R. et al. Use of doxycicline for leptospirosis after high-risk exposure in São Paulo, Brazil. **Rev. Inst. Med. Trop. São Paulo**, v. 40, n. 1, p. 59-61, 1998.

GOUVEIA, E.L.et al. Leptospirosis-associated severe pulmonar hemorrhagic syndrome, Salvador, Brazil. **Emerg. Infect. Dis.**, v. 14, n. 3, p. 505-508, 2008.

HADAD, E. et al. An outbreak of leptospirosis among israeli troops near the Jordan river. **Am. J. Trop. Med. Hyg.**, v. 74, n. 1, p. 127–131, 2006.

HARTSKEERL, R. A. Leptospirosis: current status and future trends. **Indian J. Med. Microbiol.**, v. 24, p. 309, 2006.

HERMANN STORCK, C. et al. Changes in epidemiology of leptospirosis in 2003–2004, a two El Nino Southern oscillation period, Guadeloupe archipelago, French West Indies.**Epidemiol. Infect.**, v. 136, p. 1407–1415, 2008.

HERNÁNDEZ, M.S. et al. Leptospirosis en niños de la Provincia de Ciego de Ávila, Cuba. **Rev. Soc. Bras. Med. Trop.**, v. 32, n. 2, p. 145-150, 1999.

HIN, L.Y.; LAU, T.K.; ROGERS, M.S.; CHANG, A.M. Dichotomization of continuous measurements using generalized additive modelling–application in predicting intrapartum caesarean delivery. **Stat. Med.,** v. 18, p. 1101–1110, 1999.

HOFFMEISTER, B. et al, Differences in clinical manifestations of imported vs autochtonous leptospirosis in Austria and Germany. **Am. J. Trop. Med. Hyg.**, v. 83, n. 2, p. 326-335, 2010.

HOLK, K.; NIELSEN, S.V.; RONNE, T. Human leptospirosis in Denmark 1970-1996: an epidemiological and clinical study. **Scand. J. Infect. Dis.,** v. 32, p. 533-538, 2000.

HOTEZ, P.J. Neglected infections of poverty in the United States of America. **PLoS Negl. Trop. Dis.**, v. 2, n. 6, p. e256, 2008.

HÜBENER, E. A.; Reiter, H. Beitrage zur Aetiologie der Weilschen Krankheit. **Dtsch. Med. Wschr.**, v. 41, p. 1275-1277, 1915.

HUSTON, C. D. Leptospirosis. In: **Clinical Infectious Disease**. New York: Cambridge University Press, 2008. p.1139-41.

IDO, Y.; HOKI, R. The mode of invasion of Spirochaeta icterohaemorrhagiae. **Iji Shinbun No. 931.** Nippon Gakkai Zasshi**,** v. 3, n., p. 3, 1915.

INADA, R., et al. The etiology, mode of infection, and specific therapy of Weil's disease (*Spirochaetatosisicterohaemorrhagica*).**J. Exp. Med.**, v. 23, p. 377-402, 1916.

JANSEN, A. et al. Sex differences in clinical leptospirosis in Germany: 1997 – 2005..**Clin. Infec. Dis.**, v. 44, p. e69-72, 2007.

JENA, A.B.; MOHANTY, K.C.; DEVADASAN, N. An outbreak of leptospirosis in Orissa, India: the importance of surveillance. **Trop. Med. Internat. Health**, v. 9, n. 9, p. 1016–121, 2004.

JOHN, T.J.; RAJAPPAN, K.; ARJUNAN, K.K. Communicable diseases monitored by disease surveillance in Kottayam district, Kerala state, India. **Indian J. Med. Res.**, v. 120, p. 86-93, 2004.

JOHNSON, M.A.S. et al. Environmental exposure and leptospirosis, Peru. **Emerg. Infec. Dis.**, v. 10, n. 6, p. 1016-1022, 2004.

KARANDE, S. et al. Leptospirosis in children in Mumbai slums. **Indian J. Pediatr.**, v. 69, n. 10, p .855-858, 2002.

KARIV, R. et al, The changing epidemiology of leptospirosis in Israel. **Emerg. Infec. Dis.,** v. 7, n. 6, p. 990-992, 2001.

KAWAGUCHI, L. et al. Seroprevalence of leptospirosis and risk factor analysis in flood-prone rural areas in Lao PDR. **Am. J. Trop. Med. Hyg.**, v. 78, n. 6, p. 957-961, 2008.

KEENAN, J. et al. Risk factors for clinical leptospirosis from Western Jamaica. **Am. J. Trop. Med. Hyg.**, v. 83, n. 3, p. 633-636, 2010.

KO, A.I. et al. Urban epidemic of severe leptospirosis in Brazil. **The Lancet**, v. 353, p. 820–825, 1999.

KO, A.I., GOARANT, C., PICARDEAU, M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. **Nat. Rev. Microbiol.,** v. 7, n. 10, p.736-747, 2009.

KOIZUMI, N. et al. Serological and genetic analysis of leptospirosis in patients with acute febrile illness in Kandy, Sri Lanka. **Jpn. J. Infec. Dis.**, v. 62, p. 474-475, 2009.

KUPEK, E.; FAVERSANI, M. C. S. S.; PHILIPPI, J. M. S. The relationship between rainfall and human leptospirosis in Florianópolis, Brazil, 1991-1996. **Braz. J. Infec. Dis.**, v. 4, n. 3, p. 131-134, 2000.

LACERDA, H. G. et al. Leptospirosis in a subsistence farming community in Brazil. **Trans. Royal Soc. Trop. Med. Hyg.,** v. 102, n. 12, p. 1233-1238, 2008.

LEBLEBICIOGLU, H. et al. Weil’s disease: report of 12 cases. **Scand. J. Infect. Dis.**,v. 28, p. 637-6 39, 1996.

LELIS, S. S. R. et al. Acute disseminated encephalomyelitis after leptospirosis. **Pediatr. Neurol.**, v. 40, p. 471-473, 2009.

LEVESQUE, B. Seroepidemiologic study of three zoonoses (Leptospirosis, Q Fever, and Tularemia) among trappers in Quebéc, Canada. **Clin. Diagnostic Lab. Immunol.,** v. 4, n. 2, p .496–498, 1995.

LEVETT, P.N. Leptospirosis. **Clin. Microb. Rev.**, v. 14, n. 2, p. 296-326, 2001.

LEVETT, P. N. Leptospirosis. In: MANDELL, G. L.; BENNET, J. E.; Dolin, R. **Principles and Practice of Infectious Diseases**. 6ed. Philadelfia: Elsevier, 2005. 3662p.

LEVETT, P. N., BRANCH, S. L., EDWARDS, C. N. Detection of dengue infection in patients investigated for leptospirosis in Barbados. **Am. J. Trop. Med. Hyg.**, v. 62, n. 1, p. 112–114, 2000.

LIBRATY, D. H. et al. A comparative study of leptospirosis and dengue in thai children. **PLoSNegl. Trop. Dis.**, v. 1, n. 3, p.e111, 2007.

LIMA, S. C. et al. Outbreak of human leptospirosis by recreational activity in the human in the municipality of São José dos Campos, São Paulo – Seroepidemiology study. **Rev. Inst. Med. Trop. São Paulo**, v. 32, n. 6, p. 474-479, 1990.

LIN, P. C. et al. Demographic and clinical features of leptospirosis: three-year experience in central Taiwan. **J. Microbiol. Immunol. Infect.**, v. 41, p.1 45-150, 2008.

LOMAR, A.V. et al. Leptospiroses. In: VERONESI, R., FOCACIA, R. **Tratado de Infectologia**. São Paulo: Atheneu, 2005. 1139-1148p.

MACIEL, E. A. P. et al. Household transmission of leptospira infection in urban slum communities.**PLoS Negl. Trop. Dis.**, v. 2, n. 1, p.e154, 2008.

MARCHIORI, E. et al. Clinical and imaging manifestations of hemorrhagic pulmonary leptospirosis: a state-of-the-art review, **Lung**, 2010.

MARKOTIC, A. etal.Double trouble: hemorrhagic fever with renal syndrome and leptospirosis. **Scand. J. Infec. Dis.**, v. 34, p. 221-224, 2001.

MATTHIAS, M. A. et al. Human leptospirosis caused by a new, antigenically unique leptospira associated with a *Rattus* species reservoir in the Peruvian Amazon. **PLoSNegl. Trop. Dis.**, v. 2, n. 4, e213, 2008.

MCBRIDE, A. J. et al. Leptospirosis. **Curr Opin Infect Dis**. v. 18, n. 5, p.3 76-386, 2005.

MICHALOPOULOS, A. et al. Leptospirosis in a European intensive care unit. **Scand. J. Infec. Dis.**, v. 42, p. 69-71, 2010.

MORGAN, J. et al. Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. **Clin. Infec. Dis.**, v. 34, p. 1593-1599, 2002.

MONAHAN, A.M.; MILLER, I.S.; NALLY, J.E. Leptospirosis: risks during recreational activities. **J. Appl. Microbiol.**, v. 107, n. 3, p. 707-716, 2009.

NARDONE, A. et al. Risk factors for leptospirosis in metropolitan France: Results of a national case-control study, 1999-2000. **Clin. Infec. Dis.**, v. 39, p. 751-73, 2004.

OOTEMAN, M. C.; VAGO, A. R.; KOURY, M. C. Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis. **J. Microb. Methods**, v. 65, p. 247–257, 2006.

OMS. ORGANIZAÇÃO MUNDIAL DE SAÚDE. **Human leptospirosis: Guidance for diagnosis, surveillance and control.** Genebra: OMS. 2003. 122p.

PAPPACHAN, M.J.; SHEELA, J.; ARAVINDAN, K.P. Relation of rainfall pattern and epidemic leptospirosis in the Indian state of Kerala. **J. Epidemiol. Commun. Health**, v. 58, p. 1054–1055, 2004.

PEREIRA, G.C. Habitação e infra-estrutura urbana em Salvador e região metropolitana. In: CARVALHO, I.M.M.; PEREIRA, G.C. **Como anda Salvador**. 2ª ed. Salvador: Edufba. 2008. p.137-55, 228p.

PERIC, L. et al. Human leptospirosis in eastern Croatia, 1969-2003: Epidemiological, clinical, and serological features, **Scand. J. Infec. Dis.**, v. 37, p. 738-741, 2005.

PHILLIP, R.; KING, C.; HUGHES, A.Understanding of Weil's disease among canoeists.**Br. J. Sp. Med.**, v. 26, n. 4, 1992.

ROMERO, E.C.; BERNARDO, C.C.D.M., YASUSA, P.H. Human leptospirosis: A twenty-nine year serological study in São Paulo, Brazil. **Rev. Inst. Med. Trop. São Paulo**, v. 45, n. 5., p. 245-248, 2003.

ROMERO, M.H.; SÁNCHEZ, J.A.; HAYEK, L.C. Prevalencia de anticuerpos contra *Leptospira* en población urbana humana y canina del Departamento del Tolima. **Rev. Salud Públ.**, v. 12, p. 268-275, 2010.

SARKAR, U. et al. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. **Am. J. Trop. Med. Hyg.**, v. 66, n. 5, p.605-610, 2002.

SEHGAL, S.C., SUGUNAN, A.P., VIJAYACHARI, P. Outbreak of leptospirosis after cyclone in Orissa. **Nat. Med. J. India**, v. 15, n.1 , p. 22-23, 2002.

SEGURA, E.R. et al. Clinical spectrum of pulmonary involvement in leptospirosis in a region of endemicity, with quantification of leptospiral burden. **Clin. Infect. Dis.**, v. 40, n. 3, p. 343-51, 2005.

SETHI, S. et al. Increasing trends of leptospirosis in Northern India: A clinico-epidemiological study. **PLoSNegl. Trop. Dis.**, v. 4, n. 11, p. e579, 2010.

SEVJAR, J. et al An outbreak of leptospirosis, Thailand – The importance of the laboratory. **Southeast Asian J. Trop. Med. Publ. Health**, v. 36, n. 2, p. 289-295, 2005.

SILVA, A.D.; EVANGELISTA, M.S.N. Syndromic surveillance: Etiologic study of acute febrile illness in dengue suspicious cases with negative serology. Brazil, Federal District, 2008. **Rev. Inst. Med. Trop. Sao Paulo**, v. 52, n. 5, p. 237-242, 2010.

SILVA, H.R. et al. Síndrome de meningite asséptica por enterovírus e *Leptospira*sp. em crianças de Salvador, Bahia. **Rev. Soc. Bras. Med. Trop.**, v. 35, n. 2, p. 159-165, 2002.

SLACK, A.T. et al. The epidemiology of leptospirosis and the emergence of *Leptospiraborgpetersenii*serovar*Arborea* in Queensland, Australia, 1998 – 2004. **Epidemiol. Infect.,** v. 134, p. 1217-1225, 2006.

SMYTHE, L.D. et al. A quantitative PCR (TaqMan) assay for pathogenic *Leptospira* spp. **BMC Infec. Dis.**, v. 2, p.13, 2002.

SOARES, T.S.M. et al. Spatial and seasonal analysis on leptospirosis in the municipality of São Paulo, Southeastern Brazil, 1998 to 2006. **Rev. Saúde Públ.**, v. 44, n. 2, p. 2-9, 2010.

SOUZA, A. I.; NOGUEIRA, J. M. R.; PEREIRA, M. M. Anticorpos anti-*Leptospira*em pacientes de Mato Grosso do Sul com suspeita clínica de dengue ou hepatite viral. **Rev. Soc. Bras. Med. Trop.**, v. 40, n. 4, p. 431-435, 2007.

SOUZA, V. M. M. et al. Avaliação do Sistema Nacional de Vigilância Epidemiológica da Leptospirose – Brasil, 2007. **Cad. Saúde Col.,** v. 18, p. 95 - 105, 2010.

SPICHLER, A. et al., Using death certificate reports to find severe leptospirosis cases, Brazil. **Emerg. Infec. Dis.**, v. 13, n. 10, 2007.

STERN, E.J. et al. Outbreak of leptospirosis among Adventure Race participants in Florida, 2005. **Clin. Infect. Dis.,** v. 50, n. 6, p. 843-849, 2010.

STIMSON, A. M. Note on an organism found in yellow fever tissue. **Publ .Health. Rep. Wash.,** v. 22, p. 541, 1907.

SUNBUL, M., et al. *Rattus norvegicus* acting as reservoir of *Leptospira interrogans* in the Middle Black Sea Region of Turkey, as evidenced by PCR and presence of serum antibodies to Leptospira strain. **Scand. J. Infect. Dis.**, v. 33, p. 896-888, 2001.

TANGKANAKUL, W. et al. Risk factors associated with leptospirosis in Northeastern Thailand, 1998. **Am. J. Trop. Med.**, v. 63, n. 3, p. 204-208, 2000.

TASSINARI, W.S. et al. Detection and modelling of case clusters for urban leptospirosis, **Trop. Med. Int. Health**, v. 13, n. 4, p. 503–512, 2008.

THAIPADUNGPANIT, J. et al. A dominant clone of *Leptospira interrogans* associated with an outbreak of human leptospirosis in Thailand. **PLoSNegl. Trop. Dis.,** v. 1, n. 1, p-e56, 2007.

TOYOKAWA, T., OHNISHI, M., KOIZUMI,N.Diagnosis of acute leptospirosis. **Expert Rev. Anti. Infect.Ther.,** v. 9, n. 1, p. 111-121, 2011.

TURHAN, V. et al. Leptospirosis in Istanbul, Turkey: A wide spectrum in clinical course and complications. **Scand. J. Infect. Dis.**, v. 38, p. 845-852, 2006.

UHLENHUTH, P.; FROME, W. Experimentelle Untersuchung uber die sog. **Weilsche Krankheit (ansteckende Gelbsucht). Med Klinik**, v. 44, n., p. 1202, 1264, 1375, 1915.

UN-HABITAT. **Slums of the world: The face of urban poverty in the new millennium?**Nairobi: UN-Habitat. 2003. 94p.

VALDÉS, C.M.; BREIJO, E.O.A. Comportamento de la leptospirosis humana, **Rev. Cubana Enfermer.**, v. 17, n. 3, p. 168-172, 2001.

VADO-SÓLIS, I. et al. Clinical-epidemiological study of leptospirosis in humans and reservoirs in Yucatán, México. **Rev. Inst. Med. Trop. São Paulo**, v. 44, n. 6, p. 335-340, 2002.

VANASCO, N.B. et al. Clinical characteristics and risk factors of human leptospirosis in Argentina (1999–2005), **Acta Trop.,** v. 107, p. 255–258, 2008.

VICTORIANO, A.F. et al. Leptospirosis in the Asia Pacific region. **BMC Infect. Dis.,** v. 9, n. 147, 2009.

VIEIRA, M.L.; GAMA-SIMÕES, M.J.; COLLARES-PEREIRA, M. Human leptospirosis in Portugal: a retrospective study of eighteen years. **Int. J. Infec. Dis.**, v. 10, p. 378-386, 2006.

VIJAYACHARI, P.; SUGUNAN, A.P.; SEHGAL, S.C. Evaluation of LeptoDri Dot as a rapid test for the diagnosis of leptospirosis. **Epidemiol. Infect.**, v. 129, p. 617-621, 2002.

VINETZ, J.M. Leptospirosis. **Curr.Opin. Infect. Dis.**, v. 14, n. 5, p. 527-538, 2001.

WHO. WORLD HEALTH ORGANIZATION. **Human leptospirosis: Guidance for diagnosis, surveillance and control.** Malta: World Health Organization, 2003.

WIWANITKIT, V. A note from a survey of some knowledge aspects of leptospirosis among sample of rural villagers in the highly endemic area, Thailand. **Rural Remote Health**, v. 6, p. 526-527, 2006.

YIMER ET AL. Human leptospirosis, in Ethiopia: a pilot study in Wonji. **Ethiop J. Health Dev.**, v. 18, p. 48-51, 2004.

ZAVALA-VELÁZQUEZ, J. Hemorrhagic pulmonary leptospirosis: three cases from the Yucatan peninsula, Mexico. **Rev. Soc. Bras. Med. Trop.**, v. 41, n. 4, p. 404-408, 2008.

10 Anexo 1

Impact of Environment and Social Gradient on Leptospira Infection in Urban Slums.

[Impacto do Gradiente Ambiental e Social na Infecção pela *Leptospira* em Favelas Urbanas].

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RESUMO:

A leptospirose tornou-se um problema de saúde urbana juntamente com o crescimento das áreas de ocupação subnormais que se expandiram em todo o mundo. Os esforços para identificar as intervenções para a leptospirose urbana têm sido dificultados pela falta de informações de base populacional sobre os determinantes de transmissão de *Leptospira*. **Objetivo:** Identificar a prevalência de infecção por *Leptospira* e fatores de risco para aquisição de infecção em uma área urbana de Salvador. **Métodos e Resultados:** Foi realizado um estudo tipo corte transversal com 3.171 residentes da localidade de Pau da Lima em Salvador. Anticorpos aglutinantes contra leptospira foram medidos como um marcador para a infecção prévia. Modelos de regressão de *Poisson* avaliou a associação entre infecção prévia e atributos ambientais obtidos a partir do uso de Sistemas de Informações Geográficas, indicadores de nível socioeconômico e de exposições para os indivíduos. A prevalência geral foi de 15,4 % (intervalo de confiança de 95% [IC], 14,0-16,8). Os indivíduos com evidência sorológica de anticorpos contra leptospira foram identificados em áreas irregulares na parte inferior dos vales. O risco de infecção foi associado a fatores ambientais, como residência em regiões de risco de inundação, com esgotos a céu aberto (razão de prevalência [RP] 1,42, 95%IC 1,14-1,75) e proximidade com lixo ([RP] 1,43, 95%IC 1,04-1,88), visualizar ratos ([RP] 1,32, 1,10-1,58), e a criação de galinhas ([RP] 1,26, 95%IC 1,05-1,51). Além disso, a baixa renda e raça negra ([RP] 1,25, 95%IC 1,03-1,50) foram fatores de risco independentes. O aumento de 1dólar por dia na renda domiciliar per capita foi associado com um 11% (IC 95 % 5% -18 %) diminuição para o risco de infecção **Conclusões:** as deficiências na infraestrutura de saneamento, onde habitantes desta área residem, configuram fontes ambientais de transmissão de *Leptospira*. Mesmo após o controle de fatores ambientais nas análises, as diferenças de condições socioeconômicas contribuíram de forma estatisticamente significantes para o risco de infecção, indicando que a prevenção eficaz da leptospirose precisa de abordagens com impacto nas transformações das características sociais que

produzem os resultados de saúde desiguais entre os moradores das áreas de ocupação subnormal, além de intervenções para melhoria do saneamento básico.

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PLoS NEGLECTED TROPICAL DISEASES

Impact of Environment and Social Gradient on *Leptospira* Infection in Urban Slums

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Abstract

Background: Leptospirosis has become an urban health problem as slum settlements have expanded worldwide. Efforts to identify interventions for urban leptospirosis have been hampered by the lack of population-based information on *Leptospira* transmission determinants. The aim of the study was to estimate the prevalence of *Leptospira* infection and identify risk factors for infection in the urban slum setting.

Methods and Findings: We performed a community-based survey of 3,171 slum residents from Salvador, Brazil. *Leptospira* agglutinating antibodies were measured as a marker for prior infection. Poisson regression models evaluated the association between the presence of *Leptospira* antibodies and environmental attributes obtained from Geographical Information System surveys and indicators of socioeconomic status and exposures for individuals. Overall prevalence of *Leptospira* antibodies was 15.4% (95% confidence interval [CI], 14.0–16.8). Households of subjects with *Leptospira* antibodies clustered in squatter areas at the bottom of valleys. The risk of acquiring *Leptospira* antibodies was associated with household environmental factors such as residence in flood-risk regions with open sewers (prevalence ratio [PR] 1.42, 95% CI 1.14–1.75) and proximity to accumulated refuse (1.43, 1.04–1.88), sighting rats (1.32, 1.10–1.58), and the presence of chickens (1.26, 1.05–1.51). Furthermore, low income and black race (1.25, 1.03–1.50) were independent risk factors. An increase of US$1 per day in per capita household income was associated with an 11% (95% CI 5%–18%) decrease in infection risk.

Conclusions: Deficiencies in the sanitation infrastructure where slum inhabitants reside were found to be environmental sources of *Leptospira* transmission. Even after controlling for environmental factors, differences in socioeconomic status contributed to the risk of *Leptospira* infection, indicating that effective prevention of leptospirosis may need to address the social factors that produce unequal health outcomes among slum residents, in addition to improving sanitation.

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Introduction

At present, one billion of the world's population resides in slum settlements [1]. This number is expected to double in the next 25 years [1]. The growth of large urban populations which are marginalized from basic services has created a new set of global health challenges [2,3]. As part of the Millennium Development Goals [4], a major priority has been to address the underlying poor sanitation and environmental degradation in slum communities which in turn, are the cause of a spectrum of neglected diseases which affect these populations [2,3,5].

Leptospirosis is a paradigm for an urban health problem that has emerged due to recent growth of slums [6,7]. The disease, caused by the *Leptospira* spirochete, produces life-threatening manifestations, such as Weil's disease and severe pulmonary hemorrhage syndrome for which fatality is more than 10% and 50%, respectively [7 9]. Leptospirosis is transmitted during direct contact with animal reservoirs or water and soil contaminated with their urine [8,9]. Changes in the urban environment due to expanding slum communities has produced conditions for rodent-borne transmission [6,10]. Urban epidemics of leptospirosis now occur in cities throughout the developing world during seasonal heavy rainfall and flooding [6,11 18]. There is scarce data on the burden of specific diseases that affect slum populations [2], however leptospirosis appears to have become a major infectious disease problem in this population. In Brazil alone, more than

Author Summary

Leptospirosis, a life-threatening zoonotic disease, has become an important urban slum health problem. Epidemics of leptospirosis now occur in cities throughout the developing world, as the growth of slum settlements has produced conditions for rat-borne transmission of this disease. In this prevalence survey of more than 3,000 residents from a *favela* slum community in Brazil, Geographical Information System (GIS) and modeling approaches identified specific deficiencies in the sanitation infrastructure of slum environments—open sewers, refuse, and inadequate floodwater drainage—that serve as sources for *Leptospira* transmission. In addition to the environmental attributes of the slum environment, low socioeconomic status was found to independently contribute to the risk of infection. These findings indicate that effective prevention of leptospirosis will need to address the social factors that produce unequal health outcomes among slum residents, in addition to improving sanitation.

10,000 cases of severe leptospirosis are reported each year due to outbreaks in urban centers [19], whereas roughly 3,000, 8,000 and 1,500 cases are reported annually for meningococcal disease, visceral leishmaniasis and dengue hemorrhagic fever, respectively, which are other infectious disease associated with urban poverty [20–22]. Case fatality (10%) from leptospirosis [19] is comparable to that observed for meningococcal disease, visceral leishmaniasis and dengue hemorrhagic fever (20%, 8% and 10%, respectively) in this setting [20,23,24]. Furthermore, leptospirosis is associated with extreme weather events, as exemplified by the El Niño-associated outbreak in Guayaquil in 1998 [25]. Leptospirosis is therefore expected to become an increasingly important slum health problem as predicted global climate change [26,27] and growth of the world's slum population [1] evolves.

Urban leptospirosis is a disease of poor environments since it disproportionately affects communities that lack adequate sewage systems and refuse collection services [6,10,11]. In this setting, outbreaks are often due to transmission of a single serovar, *L. interrogans* serovar Copenhageni, which is associated with the *Rattus norvegicus* reservoir [6,28–30]. Elucidation of the specific determinants of poverty which have led to the emergence of urban leptospirosis is essential in guiding community-based interventions which, to date, have been uniformly unsuccessful. Herein, we report the findings of a large seroprevalence survey performed in a Brazilian slum community (*favela*). Geographical Information System (GIS) methods were used to identify sources for *Leptospira* transmission in the slum environment. Furthermore, we evaluated whether relative differences in socioeconomic status among slum residents contributed to the risk of *Leptospira* infection, in addition to the attributes of the environment in which they reside.

Methods

Study site and population

The study was conducted in the Pau da Lima community (Figure 1A) which is situated in the periphery of Salvador, a city of 2,443,107 inhabitants [31] in Northeast Brazil. Pau da Lima is a region of hills and valleys, which was a sparsely inhabited area of Atlantic rain forest in the 1970s and subsequently transformed into a densely-populated slum settlement (Figure 1B) due to in-migration of squatters. In total, 67% of the population of Salvador and 37% of the urban population in Brazil reside in slum communities with equal or greater levels of poverty as that found in Pau da Lima [32,33].

A study site was established which comprised of four valleys in an area of 0.46 km^2 (Figure 1A). Active hospital-based surveillance found that the mean annual incidence of severe leptospirosis was 57.8 cases per 100,000 population at the study site between 1996 and 2001 (unpublished data). The study team conducted a census during visits to 3,689 households within the site in 2003 and identified 14,122 inhabitants. Households were assigned sequential numbers. A computer-based random number generator was used to select a list of 1,079 sample households from a database of all enumerated households. Eligible subjects who resided in sample households and had five or more years of age were invited to be a

Figure 1. Slum community site in the city of Salvador, Brazil. (*A*) The yellow line in the aerial photograph is the boundary of the study site in the Pau da Lima community. The map in the bottom left corner shows the location of Salvador in Brazil and the study site (red) within the city. (*B*) Photograph of the typical environment at the community study site, which shows a valley in which households is situated and the proximity of households to open sewers and refuse. (*C*) Close-up view of the orthomap used to georeference households (red and black dots) and environmental attributes, such as open sewers (blue line) and refuse deposits, for the region marked as a yellow box in *Panel A*. The red arrow represents the direction from which the photograph in *Panel B* was taken.
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study participant. Subjects were enrolled into the study between April 2003 and May 2004 according to written informed consent approved by the Institutional Review Boards of the Oswaldo Cruz Foundation, Brazilian National Commission for Ethics in Research, and Weill Medical College of Cornell University.

Household survey

The study team of community health workers, nurses and physicians conducted interviews during house visits and administered a standardized questionnaire to obtain information on demographic and socioeconomic indicators, employment and occupation, and exposures to sources of environmental contamination and potential reservoirs in the household and workplace. Responses reported by subjects were used to obtain information on race. The study team evaluated literacy according to the ability to read standardized sentences and interpret their meaning. Informal work was defined as work-related activities for which the subject did not have legal working documents. The head-of-household, defined as the member who earned the highest monthly income, was interviewed to determine sources and amounts of income for the household. Subjects were asked to report the highest number of rats sighted within the household property and the site of work-related activities. The study team surveyed the area within the household property to determine the presence of dogs, cats and chickens.

Geographical Information System (GIS) survey

An ArcView version 8.3 software system (Environmental Systems Research Institute) database was constructed with georeferenced aerial photographs and topographic maps provided by the Company for Urban Development of the State of Bahia (CONDER). Photographs of the study site, which had a scale of 1:2,000 and spatial resolution of 16cm, were taken in 2002. During the census, the study team identified households within the study site and marked their positions onto hard copy 1:1,500 scale maps (Figure 1C), which were then entered into the ArcView database. A survey was conducted during the seasonal period of heavy rainfall between April and August 2003 to geocodify the location of open sewage and rainwater drainage systems. During three time points within this period, the study team mapped the sites of open accumulated refuse and measured the area of these deposits. Mean values for areas of refuse deposits were calculated and used for the analyses.

Serological analysis

Sera were processed from blood samples collected from subjects during house visits. The microscopic agglutination test (MAT) was performed to evaluate for serologic evidence of a prior *Leptospira* infection [34]. A panel of five reference strains (WHO Collaborative Laboratory for Leptospirosis, Royal Tropical Institute, Holland) and two clinical isolates [6] were used which included *L. interrogans* serovars Autumnalis, Canicola and Copenhageni, *L. borgspetersenii* serovar Ballum, and *L. kirschneri* serovar Grippotyphosa. The use of this panel had the same performance in identifying MAT-confirmed cases of leptospirosis during surveillance in Salvador [6,16] as did the WHO recommended battery of 19 reference serovars [34]. Screening was performed with serum dilutions of 1:25, 1:50 and 1:100. When agglutination was observed at a dilution of 1:100, the sample was titrated to determine the highest titer.

Statistical methods

Information for subjects was double entered into an EpiInfo version 3.3.2 software system (Centers for Diseases Control and Prevention) database. Chi-square and Wilcoxon rank sum tests were used to compare categorical and continuous data, respectively, for eligible subjects who were and were not enrolled in the study. A P value ≤ 0.05 in two sided testing was used as the criterion for a significant difference. Preliminary analyses evaluated a range of MAT titers as criteria for prior *Leptospira* infection and found that the use of different cut-off values (1:25 1:100) identified similar associations with respect to the spatial distribution of seropositive subjects and risk factors for acquiring *Leptospira* antibodies. A titer greater or equal to 1:25 was therefore used to define the presence of *Leptospira* antibodies in the final analyses. The presumptive infecting serovar was defined as the serovar against which the highest agglutination titre was directed [34]. Crude prevalence rates were reported since age and gender-adjusted values did not differ significantly from crude values. Ninety-five percent confidence intervals (CI) were adjusted for the cluster sampling of households.

Kernel density estimation analysis was performed with a range of bandwidths (10 120 meters) to evaluate smoothed spatial distributions of subjects with *Leptospira* antibodies and all subjects. The R version 2.4.1 statistical package (R Foundation for Statistical Computing) was used to obtain estimates which were adjusted for boundary effects. The ratio of the Kernel density estimators for subjects with *Leptospira* antibodies and all subjects was measured to determine the smoothed population-adjusted risk distribution. A digital terrain model of topographic data was used (ArcGIS 3D Analyst Extension software) to obtain continuous estimates of altitude for the study area. The distances, calculated in three-dimensional space, of households to nearest open drainage systems and refuse deposits were evaluated as proxies of exposure to these sources of environmental attributes. Elevation of households with respect to the lowest point in the valley in which they were situated was used as a surrogate for flood risk. Generalized additive models (GAM) [35] were used to evaluate the functional form of the association between continuous variables and the risk of acquiring *Leptospira* antibodies. When indicated, continuous variables were categorized in multivariate analyses according to the x-intercept value observed in the plots of fitted smoothed values.

We used Poisson regression [36] to estimate the effect of demographic, socioeconomic, household and workplace-related factors on the prevalence of *Leptospira* antibodies. A Bayesian inference approach was used which incorporated two random effects in order to account for overdispersion and cluster sampling within households. This approach has been used to estimate parameters in complex models [37] and is less sensitive to sparse data [38]. Standard non-informative prior distributions were used in models which were fitted with WinBUGS version 1.4.2 (MRC Biostatistics Unit). In multivariate analysis, all variables which had a P value below 0.10 in univariate analyses were included in the initial model. In order to address co-linearity among variables, we identified sets of covariates with the high Spearman correlation coefficients (>0.3 or <-0.3). Highly correlated variables were aggregated in a single variable when indicated, and evaluated in the model. The final model was obtained which used backward variable selection with an inclusion rule of P value <0.05.

Results

Among 3,797 eligible residents from the slum community site, 3,171 (84%) were enrolled in the study. Study subjects had a higher proportion of females (56% of 3,171 subjects versus 37% of 626 subjects, respectively; $P<0.05$) and younger mean age (25.8 ± 15.2 versus 28.1 ± 14.6 years, respectively; $P<0.05$) than eligible residents who did not participate in the study. The kernel distribution of enrolled subjects according to place of residence was similar on visual inspection to that of residents who did not participate (data not shown). The majority (85%) of subjects were squatters who did not have legal title to their domiciles. Subjects belonged to mostly mixed (*pardo*, 66%) or black (28%) racial groups. Median household per capita income for study subjects was US$ 1.30 per day. Among the subjects, 76% had not completed elementary school education and 23% were illiterate. Among 2,077 subjects ≥18 years of age, 77% did not have formal employment and 35% engaged in informal work.

Among the 3,171 subjects, 489 had *Leptospira* agglutinating antibodies, as determined by the presence of MAT titer ≥1:25 (Figure 2). Highest titers were directed against *L. interrogans* serovar Copenhageni in 436 (89.2%) of the 489 subjects with *Leptospira* antibodies. For the 22 subjects (4.5%) who had highest titers against two or more serovars, agglutination reactions recognized Copenhageni as one of the serovars. Copenhageni was the predominant serovar (88–100%) recognized for the range of highest reciprocal titers (Figure 2).

The overall prevalence of *Leptospira* antibodies was 15.4% (95% CI 14.0–16.8). The crude prevalence among enrolled subjects was not significantly different from the prevalence (15.9%, 95% CI 14.6–17.1) which was adjusted for the age and gender distribution of eligible subjects in the study population. Prevalence was highest among adolescents and adults (16.2% and 21.2% for age groups 15–24 and >44 years, respectively). However, 8.3% (95% CI 6.2–10.5) of children 5–14 years of age had evidence for a prior exposure to *Leptospira*. The prevalence was higher in males than females (17.8 versus 13.6%, respectively; PR 1.32, 95% CI 1.10–1.57) (Table 1). Similar associations with age and gender were observed when MAT titers of ≥1:50 and ≥1:100 were used to define subjects with *Leptospira* antibodies.

Panels A and B in Figure 3 show smoothed spatial distributions of subjects with *Leptospira* antibodies and all subjects, respectively, according to place of residence. The population-adjusted distribution (Figure 3C) showed that risk of acquiring *Leptospira* antibodies clustered in areas occupied by squatters at the bottom of valleys (Figure 3D). Similar spatial distributions were observed in analyses that used higher titer values to define subjects with *Leptospira* antibodies (Figure S1).

Univariate analysis found the risk of acquiring *Leptospira* antibodies to be associated with increasing age, male gender, indicators of low socioeconomic level, occupations that entail contact with contaminated environments, informal work, time of residence in the study household, and environmental attributes and the presence of reservoirs in the household (Table 1). Significant risk associations were not found for formal employment and reported sighting of rats in the workplace environment. Open rainwater drainage structures and refuse deposits were distributed throughout the site; yet open sewers were more frequently encountered at the bottom of valleys (Figure 3). The distance of household to the nearest open sewer was a risk factor, whereas a significant association was not observed for distance to an open rainwater drainage system.

GAM analysis showed that the risk of acquiring *Leptospira* antibodies had an inverse linear association with the distance of the subject's household to an open sewer and elevation of the household from the lowest point in the valley, a proxy for flood risk (Figure 4). Increased risk was observed among subjects who resided less than a threshold distance of 20 meters to these attributes (Figure 4B and C). The risk of acquiring *Leptospira* antibodies had an inverse non-linear association with distance of the subject's household to an open refuse deposit (results not shown). We explored a range of dichotomization criteria and

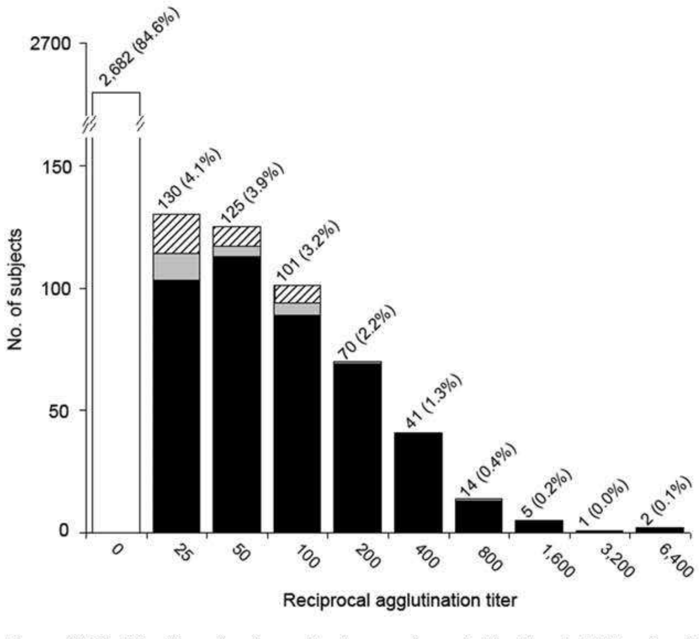

Figure 2. Distribution of reciprocal microscopic agglutination test titers for 3,171 subjects from the slum community site. Labels above the bars indicate the number of subjects (% of total), according to their highest reciprocal titer. The open bar represents seronegative subjects. Subjects with highest reciprocal titres against *L. interrogans* serovar Copenhageni, multiple serovars and serovars other than Copenhageni are shown as black bars, grey bars and crosshatched bars, respectively.
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Table 1. Risk factors for *Leptospira* antibodies among subjects at the slum community site.

Variables	*Leptospira* antibodies		PR (95% CI)	
	Yes (n = 489)	No (n = 2,682)	Univariate[a]	Multivariate[b]
	No. (%) or median (IQR)[c]			
Demographic				
Age, years				
05–14	71 (15)	781 (29)	1.00	1.00
15–24	136 (28)	704 (26)	1.98 (1.47–2.61)	2.02 (1.50–2.69)
25–34	122 (25)	524 (20)	2.31 (1.71–3.07)	2.54 (1.86–3.41)
35–44	73 (15)	350 (13)	2.11 (1.50–2.88)	2.24 (1.59–3.08)
≥45	87 (18)	323 (12)	2.60 (1.88–3.51)	2.92 (2.10–4.00)
Male gender	247 (51)	1140 (43)	1.32 (1.10–1.57)	1.38 (1.14–1.64)
Socioeconomic indicators				
Black race[d]	169 (35)	724 (27)	1.35 (1.11–1.62)	1.25 (1.03–1.50)
Household per capita income, US$/day	1.14 (0.39–1.88)	1.30 (0.61–2.20)	0.91 (0.85–0.97)[e]	0.89 (0.82–0.95)[e]
Did not complete primary school	394 (81)	2018 (75)	1.32 (1.04–1.65)	-
Household factors				
Time of residence in household, years	8 (3–17)	7 (3–12)	1.02 (1.01–1.03)[e]	-
Level above lowest point in valley, meters	18.78 (8.59–31.05)	24.71 (13.00–36.04)	0.99 (0.98–0.99)[e]	-
Distance from an open sewer, meters	14.95 (7.34–31.00)	21.04 (8.99–38.11)	0.99 (0.99–1.00)[e]	-
Distance of household from an open sewer/lowest point in valley				
≥20 m/≥20 m	158 (32)	1198 (45)	1.00	1·00
≥20 m/<20 m	38 (8)	211 (8)	1.32 (0.89–1.83)	1.19 (0.81–1.67)
<20 m/≥20 m	73 (15)	360 (13)	1.46 (1.09–1.91)	1.30 (0.97–1.71)
<20 m/<20 m	220 (45)	913 (34)	1.68 (1.36–2.05)	1.42 (1.14–1.75)
Distance from an open refuse deposit, meters	60.59 (38.48–107.54)	64.90 (42.56–103.16)	1.00 (1.00–1.00)[e]	-
<20 meters from open refuse deposit	51 (10)	174 (6)	1.53 (1.12–2.02)	1.43 (1.04–1.88)
Vegetation[f]	373 (76)	1,822 (68)	1.45 (1.17–1.79)	-
Reservoirs present in household				
Sighting of >2 rats	256 (52)	1039 (39)	1.60 (1.33–1.91)	1.32 (1.10–1.58)
Dog	231 (47)	1028 (38)	1.36 (1.14–1.62)	-
Chicken	227 (46)	988 (37)	1.40 (1.17–1.66)	1.26 (1.05–1.51)
Cat	106 (22)	406 (15)	1.44 (1.15–1.77)	-
Work-related exposures				
Informal work	157 (32)	637 (24)	1.42 (1.17–1.71)	-
Contact with contaminated environment[g]	83 (17)	284 (11)	1.57 (1.22–1.96)	-
Risk occupation[h]	49 (10)	127 (5)	1.90 (1.37–2.51)	-

[a]Univaritate prevalence ratios (PR) and 95% confidence intervals (CI) are shown for variables which were significant ($P<0.05$) in the univariate analyses.
[b]Multivariate PR and 95% CI are shown for covariates which were included in the final best fit Poisson regression model.
[c]Numbers and percentages are shown for categorical variables. Median and interquartile range (IQR) are shown for continuous variables of per capita household income, time of residence in study household, level above lowest point in valley and distance from an open sewer and refuse deposit.
[d]Data is missing for two non-infected subjects.
[e]PR and 95% CI are shown for continuous data.
[f]Data is missing for one infected and two non-infected subjects.
[g]Reported exposure to mud, refuse, flooding water or sewage water in the workplace.
[h]Occupation as construction worker, refuse collector or mechanic, which is associated with a workplace environment characterized by high rat infestation.
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found significant risk associations when subjects resided less than 20 meters from an open refuse deposit (Table 1). This association was not influenced by the size of the refuse deposit. Subjects who reported sighting two or more rats in the household environment had increased risk of acquiring *Leptospira* antibodies (Figure 4D). Household per capita income had an inverse linear association with the presence of *Leptospira* antibodies (Figure 4A). Of note, the distance of the household to an open sewer was highly correlated (Spearmen correlation coefficient = 0.71) with household elevation (Figure S2A) since open sewers drain into the bottom of valleys. An aggregate variable, distance of household located less than 20 meters from an open sewer and lowest point in a valley, was therefore used to examine the association between open sewer and flood-related exposure and infection risk (Table 1). In contrast household per capita income was not highly correlated (Spearmen correlation coefficient = 0.16) with the elevation of the household (Figure S2B).

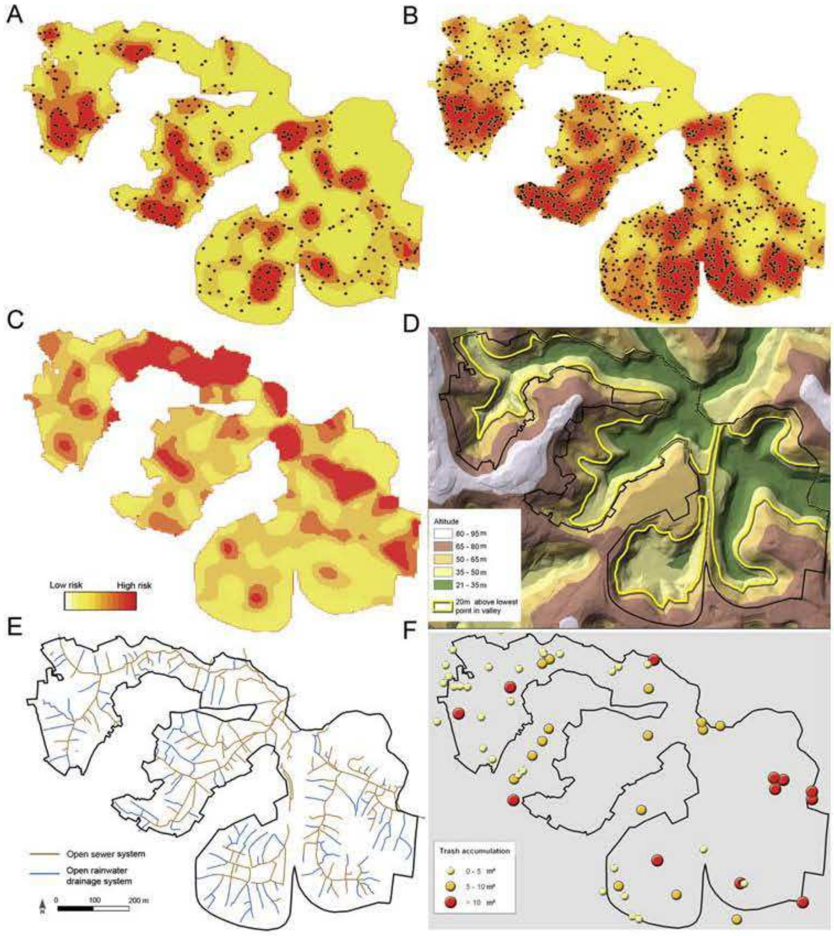

Figure 3. Spatial distribution of subjects with *Leptospira* antibodies and all enrolled subjects, according to place of residence, and environmental attributes of the community site. *Panels A* and *B* show the smoothed Kernel density distribution of subjects with *Leptospira* antibodies (N = 489) and all (N = 3,171) subjects, respectively, according to place of residence. The yellow-to-red gradient represents increasing density in smoothing analyses which used 40 meters as the bandwidth. Black circles show the location of subject households. *Panel C* shows the distribution of the population-adjusted Kernel density estimator for subjects with *Leptospira* antibodies which was calculated as the ratio of the estimators for subjects with *Leptospira* antibodies and all subjects. *Panel D* shows a topographic map generated by the digital terrain model. The yellow line is the level that is 20 meters above the lowest point in the four valleys within the community site. *Panels E* and *F* show the distribution of, respectively, open rainwater and sewage drainage systems and accumulated refuse according to size (m^2).
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Multivariate analyses found that the risk for acquiring *Leptospira* antibodies was associated with exposures in the household environment and not in the workplace setting (Table 1). Subjects who resided less than 20 meters from an open sewer and the lowest point in the valley had a 1.42 times (95% CI 1.14–1.75) increased risk for acquiring *Leptospira* antibodies than those who lived 20 meters or more from these attributes. Residence less than 20 meters from accumulated refuse was associated with a 1.43 times (95% CI 1.04–1.88) increased risk. Sighting of two or more rats and presence of chickens, a marker for rat infestation, in the household were significant reservoir-associated risk factors. After controlling for age, gender and significant environmental exposures, indicators of low socioeconomic level, household per capita income (PR 0.89 for an increase of US$1.00 per day, 95% CI 0.82–0.95) and black race (PR 1.25, 95% CI 1.03–1.50) were risk factors for acquiring *Leptospira* antibodies (Table 1).

Discussion

Efforts to identify interventions for urban leptospirosis have been hampered by the lack of population-based information on transmission determinants. In this large community-based survey of a slum settlement in Brazil, we found that 15% of the residents had serologic evidence for a prior *Leptospira* infection. The prevalence rate of *Leptospira* antibodies in the study slum community was similar to that (12%) found in a city-wide survey performed in Salvador [39]. Risk factors for acquiring *Leptospira* antibodies were associated with exposures in the household environment. Interventions therefore need to target the environmental sources of transmission - open sewers, flooding, open refuse deposits and animal reservoirs - in the places where slum inhabitants reside. After controlling for the influence of poor environment, indicators of low socioeconomic status were found to be independently associated with the risk of

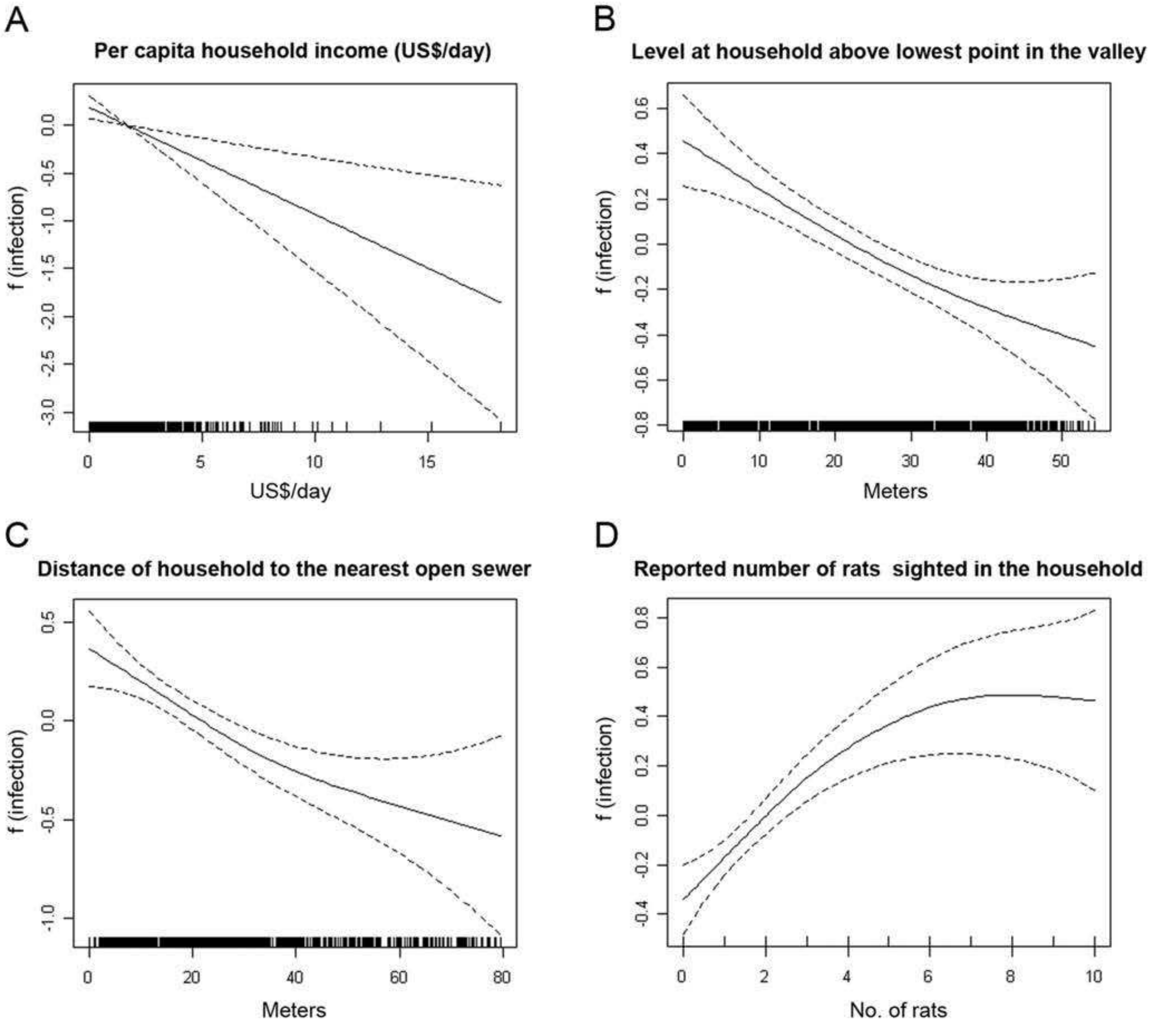

Figure 4. Generalized additive models (GAM) of the association between the risk of acquiring *Leptospira* antibodies and continuous variables of (*A*) per capita daily household income, (*B*) level of household in meters above the lowest point in valley, and (*C*) distance in meters to the nearest open sewer, and (*D*) reported number of rats sighted in the household environment. The coefficient, f(infection), in the GAM model is a measure for the risk of acquiring *Leptospira* antibodies. In *Panels A, B, C* and *D*, the x axis intercept values, where f(infection) equals zero, were US$1.70/day, 22 meters, 22 meters and 2 rats, respectively.
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acquiring *Leptospira* antibodies. This finding suggests that in slum communities with overall high levels of absolute poverty, relative differences in socioeconomic level contribute to unequal outcomes for leptospirosis.

Leptospirosis has been traditionally considered an occupational disease, since work-related activities are frequently identified as risk exposures [9]. However slum inhabitants reside in close proximity to animal reservoirs and environmental surface waters which contain *Leptospira* [10]. We previously found that *Leptospira* infection clusters within households in slum communities in Salvador [40]. In this study, we found that after controlling for confounding, significant risk exposures were those associated with the household environment rather than workplace. As a caveat, interview-elicited responses were used to evaluate work-related exposures since GIS surveys were not performed at the sites where subjects worked. It is possible that slum residents may have had work-related risk exposures which were not detected by our survey. Nevertheless, our findings support the conclusion that the slum household is an important site for *Leptospira* transmission and provides the rationale for interventions that target risk exposures in this environment.

The study's findings indicate that the domestic rat was the principal reservoir for *Leptospira* transmission in the study community. Highest agglutination titers among 89% of the subjects were directed against *L. interrogans* serovar Copenhageni, the serovar associated with the *R. norvegicus* reservoir. Reported sighting of rats is considered to be an unreliable marker of rat infestation. However we found that the number of rats sighted by residents was correlated with their risk of acquiring *Leptospira* antibodies (Figure 4D), indicating that rat sightings may be a useful marker of infection risk in slum communities where inhabitants are accustomed to the presence of rats. Although dogs were not found to be a risk factor, detailed investigations of *Leptospira* carriage in urban reservoirs need to be performed. Of note, the presence of chickens in households was a risk factor, although they in of themselves are not reservoirs. This association may reflect a rat-related exposure not accounted for by reported sightings, since rats are attracted to chicken feed and waste. Raising chickens is a widespread practice in slum communities-48% (519) of the 1079 study households raised chickens. Control of rodent reservoir populations may therefore need to incorporate measures that directly address this practice.

Our findings confirm hypotheses raised by previous ecologic studies [6,10,11] that infrastructure deficiencies related to open sewers, flooding and open refuse deposits are transmission sources for leptospirosis in the slum environment. Furthermore, there appears to be defined areas of risk associated with open sewers and refuse deposits, which serve as habitats and sources of food for rats. Home range radius of the domestic rat varies from 30 150 meters [41,42], but home range use decreases from the centre to the edge. GAM analysis demonstrated that slum residents had a positive risk for acquiring *Leptospira* antibodies when households were situated within 20 meters from open sewers and refuse deposits. In addition, infection risk increased as distances from an open sewer or refuse deposit decreased, suggesting that households which are situated closer to these foci have a higher degree of environmental contamination with *Leptospira* and inhabitants of these households are exposed to higher inoculum doses during infection. Molecular approaches to quantify *Leptospira* in environmental samples [10] will be useful in answering this question and guiding recommendations for environmental decontamination and barrier control measures which can be implemented in slum communities.

In addition, GAM analysis found that residents had positive risk for *Leptospira* infection when their households were situated within 20 meters from the lowest point in the valley (Figure 4B). In Salvador [6,12,16,40] and other urban centers [11,13,15,17,18], outbreaks of leptospirosis occur during heavy rainfall and flooding events. Slum communities are built on the poor land quality and often in areas susceptible to frequent flooding. At the study site and other slum settlements in Salvador, the water table rises up to one meter during flooding events because of inadequate rainwater drainage and blockage of drainage systems with silt and refuse. The finding that subjects had increased infection risk when their households were located within 20 meters from the lowest point in the valley suggests that this distance was a proxy for the degree of contact which residents encounter flood-related exposures in the peri-domiciliary environment.

We found that in addition to attributes of the environment where slum inhabitants reside, low per capita household income and black race, an indicator of health inequality in Brazil [43,44], were independent risk factors for *Leptospira* infection. The social gradient in health is a widespread phenomenon [45,46]. Our findings, although not unexpected, are noteworthy since they suggest that differences in status contribute to unequal health outcomes in a slum community where the household per capita income was less than US$1 per day for 44% of the inhabitants. Although errors in the measurement of risk exposures and residual confounding were a possibility, the strength of the association indicates a role for social determinants in *Leptospira* transmission. These factors may relate to risky behaviors, such as cleaning open sewers after flooding events, or limited use of protective clothing which reduce the risk of abrasions that facilitate entry of the *Leptospira* spirochete [47]. Low status and lack of access to amenities and social support are features of disadvantaged communities [45] which conceivably influence risk behaviors for leptospirosis. Further research is needed to evaluate the role of social factors such that effective interventions, including health education, can be implemented at the community level.

A limitation of our study was the cross-sectional design which used serologic evidence for a prior *Leptospira* infection as the outcome. The MAT is the standard assay used in prevalence surveys [9], yet there is not an established titer criterion for defining seropositive reactions. We previously found that a MAT titer of ≥1:25 was a specific marker for prior *Leptospira* infection among slum residents from Salvador and when applied, identified household clustering of infection risk [40]. In this study, cutoff titers from 1:25 and above identified similar risk associations. In Salvador, leptospirosis is due to transmission of a single agent, *L. interrogans* serovar Copenhageni [6,28]. Titers of 1:25, as well as higher titers, were directed against this serovar (Figure 1), indicating that this cutoff was a specific and more sensitive criteria for identifying prior infections in a region where a single serovar agent is circulating. In the study, there were more men and younger subjects among non-participating subjects than participating subjects. Crude prevalence was not different from the prevalence of *Leptospira* antibodies which was adjusted by the age and gender distribution of the overall study population, indicating that differences between participating and non-participating subjects may not have introduced a significant bias in the estimates. Infections may have occurred up to five years prior to the survey since agglutinating antibodies may persist for this period [48,49]. Major interventions to improve basic sanitation were not implemented in the study community, yet the possibility that environmental exposures were modified over time can not be excluded. Migration may have affected our ability to estimate prevalence and risk associations. An on-going cohort investigation of subjects enrolled in this study found that the annual out-migration rate is approximately 12% (unpublished data). The study's findings therefore need to be confirmed in prospective studies.

We found that *Leptospira* transmission was due to the interaction of factors associated with climate, geography and urban poverty. Since the study was performed in a single community in Salvador, Brazil, our findings may not be generalizable to other slum settings. However, a large proportion of the world's slum population resides in tropical climates similar to that in Salvador. Moreover, similar conditions of poverty and environmental degradation encountered at the study site (Figure 1B) are found in many slum settlements. In Brazil, 37% of the urban population resides in slums with equal or greater levels of poverty as found in the study community [33]. Our findings may therefore be relevant to other slum communities where leptospirosis is endemic and have increasing significance as global climate change [26,27] and growth of the world's slum population occur in the future [1,33].

The infrastructure deficiencies which were found to be transmission factors for *Leptospira* in this study can be readily addressed by improving sanitation in slum communities. Investment in sanitation is a cost-effective health intervention [50,51]. In Salvador, a city-wide sanitation program (*Bahia Azul*) was recently shown to have a major beneficial impact for diarrheal disease [52]. However, as frequently encountered with large-scale sanitation projects, the *Bahia Azul* program did not provide coverage to the study community and many of the slum settlements in the city's periphery. Equitable access to improved sanitation is therefore essential in reducing the burden of the large number of environmentally-transmitted infectious diseases, including leptospirosis, which affects slum populations. Furthermore, the finding that the social gradient within slum communities, in addition to the unhealthy environment, contributes to the risk of *Leptospira* infection suggests that prevention of urban leptospirosis will need to combine approaches for improving sanitation with approaches that identify and address the social determinants which produce unequal health outcomes.

Supporting Information

Figure S1 Smoothed Kernel density distribution of subjects with microscopic agglutination test titres of ≥1:25 (*A*), ≥1:50 (*B*) and ≥1:100 (*C*), according to place of residence at the study site. The yellow-to-red gradient represents increasing density in smoothing analyses which used 40 meters as the bandwidth.
Found at: doi:10.1371/journal.pntd.0000228.s001 (2.61 MB TIF)

Figure S2 Spot plots of the relationship between elevation of household level from the lowest point in valley and distance of the household to the nearest open sewer (*A*) and household per capita daily income (*B*). Closed and open dots represent houses with at least one seropositive subject and without a seropositive subject, respectively.
Found at: doi:10.1371/journal.pntd.0000228.s002 (1.02 MB TIF)

Alternative Language Abstract S1 Abstract translated into Portuguese by Dr. Guilherme Ribeiro.
Found at: doi:10.1371/journal.pntd.0000228.s003 (0.03 MB DOC)

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Author Contributions

Conceived and designed the experiments: RBR RF FS SM AM RRR MR AK. Performed the experiments: RBR RF FS SM AM AQ AS MR AK. Analyzed the data: RBR GR RF RRR WT MC AK. Contributed reagents/materials/analysis tools: AK. Wrote the paper: RBR GR RF RRR MC AK. Reviewed and revised the final version of the manuscript: RBR GR RF FS AM AQ AS RRR WT MC MR.

References

1. United Nations Human Settlements Programme (2003) The challenge of slums: Global report on human settlements 2003. London: Earthscan Publications Ltd. 310 p.
2. Riley LW, Ko AI, Unger A, Reis MG (2007) Slum health: Diseases of neglected populations. BMC Int Health Hum Rights 7: 2.
3. Sclar ED, Garau P, Carolini G (2005) The 21st century health challenge of slums and cities. Lancet 365: 901 903.
4. The General Assembly of United Nations (2000) United Nations Millennium Declaration. Available: http://www.un.org/millennium/. Accessed 11 Mar 2008.
5. Bartram J, Lewis K, Lenton R, Wright A (2005) Focusing on improved water and sanitation for health. Lancet 365: 810 812.
6. Ko AI, Reis MG, Ribeiro Dourado CM, Johnson WD Jr, Riley LW (1999) Urban epidemic of severe leptospirosis in Brazil. Salvador Leptospirosis Study Group. Lancet 354: 820 825.
7. McBride AJ, Athanazio DA, Reis MG, Ko AI (2005) Leptospirosis. Curr Opin Infect Dis 18: 376 386.
8. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, et al. (2003) Leptospirosis: A zoonotic disease of global importance. Lancet Infect Dis 3: 757 771.
9. Levett PN (2001) Leptospirosis. Clin Microbiol Rev 14: 296 326.
10. Ganoza CA, Matthias MA, Collins-Richards D, Brouwer KC, Cunningham CB, et al. (2006) Determining risk for severe leptospirosis by molecular analysis of environmental surface waters for pathogenic *Leptospira*. PLoS Med 3: e308. doi:10.1371/journal.pmed.0030308.
11. Barcellos C, Sabroza PC (2000) Socio-environmental determinants of the leptospirosis outbreak of 1996 in western Rio de Janeiro: A geographical approach. Int J Environ Health Res 10: 301 313.
12. Caldas EM, Sampaio MB (1979) Leptospirosis in the city of Salvador, Bahia, Brazil: A case-control seroepidemiologic study. Int J Zoonoses 6: 85 96.
13. Karande S, Kulkarni H, Kulkarni M, De A, Varaiya A (2002) Leptospirosis in children in Mumbai slums. Indian J Pediatr 69: 855 858.
14. LaRocque RC, Breiman RF, Ari MD, Morey RE, Janan FA, et al. (2005) Leptospirosis during dengue outbreak, Bangladesh. Emerg Infect Dis 11: 766 769.
15. Romero EC, Bernardo CC, Yasuda PH (2003) Human leptospirosis: A twenty-nine-year serological study in Sao Paulo, Brazil. Rev Inst Med Trop Sao Paulo 45: 245 248.
16. Sarkar U, Nascimento SF, Barbosa R, Martins R, Nuevo H, et al. (2002) Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. Am J Trop Med Hyg 66: 605 610.
17. Tassinari W de S, Pellegrini D da C, Sabroza PC, Carvalho MS (2004) [Spatial distribution of leptospirosis in the city of Rio de Janeiro, Brazil, 1996 1999]. Cad Saude Publica 20: 1721 1729.
18. Kupek E, de Sousa Santos Faversani MC, de Souza Philippi JM (2000) The relationship between rainfall and human leptospirosis in Florianopolis, Brazil, 1991 1996. Braz J Infect Dis 4: 131 134.
19. Health Surveillance Secretary, Brazilian Ministry of Health (2007) [Leptospirosis case notification records, Brazil].
20. Health Surveillance Secretary, Brazilian Ministry of Health (2008) [Dengue epidemiological report, January December 2007]. Available: http://portal.saude.gov.br/portal/arquivos/pdf/boletim_dengue_010208.pdf. Accessed 11 Mar 2008.
21. Health Surveillance Secretary, Brazilian Ministry of Health (2007) [Confirmed cases of meningococcal disease, Brazil, major regions and federal units, 1990 2006]. Available: http://portal.saude.gov.br/portal/arquivos/pdf/tabela_meningites_brasil.pdf. Accessed 11 Mar 2008.
22. Health Surveillance Secretary, Brazilian Ministry of Health (2007) [Visceral leishmaniasis case notification records, Brazil].
23. Health Surveillance Secretary, Brazilian Ministry of Health (2007) [Meningococcal disease deahts, Brazil, major regions and federal units, 1990 2006]. Available: http://portal.saude.gov.br/portal/arquivos/pdf/tabela_obitos_dm_brasil.pdf. Accessed 11 Mar 2008.
24. Health Surveillance Secretary, Brazilian Ministry of Health (2007) [Visceral leishmaniasis case fatality rate, Brazil, major regions and federal units, 2000 2006]. Available: http://portal.saude.gov.br/portal/arquivos/pdf/tabela_lv_letalidade.pdf. Accessed 11 Mar 2008.
25. Ministerio de Salud Pública, Organización Panamericana de la Salud (1998) El fenómeno El Niño en Ecuador. In: Organización Panamericana de la Salud, editor. El Niño, 1997 1998. Washington, D.C.: Organización Panamericana de la Salud. pp 175 230.
26. Epstein PR (1999) Climate and health. Science 285: 347 348.
27. Patz JA, Campbell-Lendrum D, Holloway T, Foley JA (2005) Impact of regional climate change on human health. Nature 438: 310 317.
28. Barocchi MA, Ko AI, Ferrer SR, Faria MT, Reis MG, et al. (2001) Identification of new repetitive element in *Leptospira interrogans* serovar copenhageni and its application to PCR-based differentiation of *Leptospira* serogroups. J Clin Microbiol 39: 191 195.
29. Pereira MM, Matsuo MG, Bauab AR, Vasconcelos SA, Moraes ZM, et al. (2000) A clonal subpopulation of *Leptospira interrogans* sensu stricto is the major cause of leptospirosis outbreaks in Brazil. J Clin Microbiol 38: 450 452.
30. Romero EC, Yasuda PH (2006) Molecular characterization of *Leptospira* sp. strains isolated from human subjects in Sao Paulo, Brazil using a polymerase chain reaction-based assay: A public health tool. Mem Inst Oswaldo Cruz 101: 373 378.
31. Instituto Brasileiro de Geografia e Estatística (2002) Censo demográfico 2000 - resultados do universo. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística.
32. Secretaria de Combate à Pobreza e às Desigualdades Sociais/SECOMP (2005) Mapamento da pobreza em áreas urbanas do estado da Bahia. CD-ROM 2005.
33. UN-HABITAT (2003) Slums of the world: The face of urban poverty in the new millennium? Nairobi: UN-HABITAT. 94 p.
34. World Health Organization (2003) Human leptospirosis: Guidance for diagnosis, surveillance and control. Malta: World Health Organization. 122 p.
35. Hin LY, Lau TK, Rogers MS, Chang AM (1999) Dichotomization of continuous measurements using generalized additive modelling application in predicting intrapartum caesarean delivery. Stat Med 18: 1101 1110.

36. Barros AJ, Hirakata VN (2003) Alternatives for logistic regression in cross-sectional studies: An empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol 3: 21.
37. Ashby D (2006) Bayesian statistics in medicine: A 25 year review. Stat Med 25: 3589 3631.
38. Greenland S, Schwartzbaum JA, Finkle WD (2000) Problems due to small samples and sparse data in conditional logistic regression analysis. Am J Epidemiol 151: 531 539.
39. Dias JP, Teixeira MG, Costa MC, Mendes CM, Guimaraes P, et al. (2007) Factors associated with *Leptospira* sp infection in a large urban center in Northeastern Brazil. Rev Soc Bras Med Trop 40: 499 504.
40. Maciel EAP, Carvalho ALF, Nascimento SF, Matos RB, Gouveia EL, et al. (2008) Household transmission of *Leptospira* infection in urban slum communities. PLoS Negl Trop Dis 2: e154. doi:10.1371/journal.pntd.0000154.
41. Jackson WB (1982) Norway rat and allies. In: Chapman JA, Feldhamer GA, eds. Wild mammals of North America. Baltimore: The Johns Hopkins University Press. pp 1077 1088.
42. Nowak RM (1991) Walker's mammals of the world. Baltimore: The John Hopkins University Press.
43. Barros FC, Victora CG, Horta BL (2001) Ethnicity and infant health in Southern Brazil. A birth cohort study. Int J Epidemiol 30: 1001 1008.
44. Travassos C, Williams DR (2004) The concept and measurement of race and their relationship to public health: A review focused on Brazil and the United States. Cad Saude Publica 20: 660 678.
45. Marmot M (2001) Inequalities in health. N Engl J Med 345: 134 136.
46. Marmot M (2005) Social determinants of health inequalities. Lancet 365: 1099 1104.
47. Phraisuwan P, Whitney EA, Tharmaphornpilas P, Guharat S, Thongkamsamut S, et al. (2002) Leptospirosis: Skin wounds and control strategies, Thailand, 1999. Emerg Infect Dis 8: 1455 1459.
48. Cumberland P, Everard CO, Wheeler JG, Levett PN (2001) Persistence of anti-leptospiral IgM, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979 1989. Eur J Epidemiol 17: 601 608.
49. Lupidi R, Cinco M, Balanzin D, Delprete E, Varaldo PE (1991) Serological follow-up of patients involved in a localized outbreak of leptospirosis. J Clin Microbiol 29: 805 809.
50. Hutton G, Haller L, Bartram J (2007) Global cost-benefit analysis of water supply and sanitation interventions. J Water Health 5: 481 502.
51. Laxminarayan R, Chow J, Salles S, Maslen P (2006) Intervention cost-effectiveness: Overview of general messages. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M et al., eds. Disease control priorities in developing countries. New York: Oxford University Press. pp 35 86.
52. Barreto ML, Genser B, Strina A, Teixeira MG, Assis AM, et al. (2007) Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: Assessment by two cohort studies. Lancet 370: 1622 1628.