FIOCRUZ

FUNDAÇÃO OSWALDO CRUZ
CENTRO DE PESQUISAS GONÇALO MONIZ

CURSO DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA EM SAÚDE E MEDICINA INVESTIGATIVA

TESE DE DOUTORADO

ESTUDO LONGITUDINAL DA LEPTOSPIROSE URBANA: INVESTIGAÇÃO DE FATORES DE RISCO PARA INFECÇÃO E PARA O DESENVOLVIMENTO DE FORMAS GRAVES APÓS A INFECÇÃO

GUILHERME DE SOUSA RIBEIRO

Salvador - Bahia – Brasil
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Tese submetida à Coordenação da Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa, Fundação Oswaldo Cruz, para a obtenção do Título de Doutor.

Orientador: Dr. Mitermayer Galvão dos Reis
Co-orientador: Dr. Albert Icksang Ko

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Ficha Catalográfica elaborada pela Biblioteca do
Centro de Pesquisas Gonçalo Moniz / FIOCRUZ - Salvador - Bahia.

Ribeiro, Guilherme de Sousa
R484e Estudo longitudinal da leptospirose urbana: investigação de fatores de risco para infecção e para o desenvolvimento de formas graves após a infecção [manuscrito] / Guilherme de Sousa Ribeiro. - 2008.
70 f.; 30 cm

Datilografado (fotocópia).

Tese (doutorado) – Centro de Pesquisa Gonçalo Moniz, Fundação Oswaldo Cruz. 2008.
Orientador: Prof. Mitermayer Galvão dos Reis, Laboratório de Patologia e Biologia Molecular.

1. Leptospirose. 2. *Leptospira*. 3. Epidemiologia. 4. Fatores de risco. 5. Distribuição por idade e sexo. I.Título.

CDU 616.986:614.4

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A Carine, minha esposa e companheira,

pela compreensão e amor durante a caminhada.

Aos meus pais, irmãs e amigos, pelo apoio e incentivo.

AGRADECIMENTOS

AGRADECIMENTO ESPECIAL aos meus orientadores e amigos Mitermayer e Albert, que nesses dez anos de convivência me ensinaram fundamentos da ciência e da pesquisa: compromisso social, ética, dedicação e paciência.

AGRADECIMENTO ESPECIAL a todos os estudantes e membros do grupo que contribuíram para a construção destes resultados.

AGRADECIMENTO ESPECIAL aos pacientes com leptospirose e aos moradores de Pau da Lima que se voluntariaram para participarem deste trabalho.

Aos colegas e amigos da FIOCRUZ com quem aprendo um pouco a cada dia.

Aos familiares e amigos que me incentivam a ir em frente.

RESUMO

A leptospirose grave emergiu como um importante problema de saúde pública urbana devido à expansão das favelas em todo o mundo. Medidas de prevenção são necessárias para reduzir a morbidade e mortalidade associadas à doença. Entretanto, a ausência de informações populacionais sobre determinantes para infecção e para o desenvolvimento de doença grave após a infecção tem limitado a implementação de intervenções. Nossos objetivos são identificar fatores de risco para infecção pela *Leptospira* em uma comunidade urbana e determinar a influência da idade e do sexo sobre o risco de infecção e progressão da doença após a infecção. Em um estudo de corte transversal realizado na comunidade de Pau da Lima, Salvador, nós identificamos que 15% dos 3.171 residentes inclusos no estudo apresentavam evidência sorológica para infecção prévia pela *Leptospira*. Uso de Sistema de Informação Geográfica e de abordagens de modelagem estatística identificaram deficiências de saneamento – esgotos abertos, áreas de risco para alagamento e acúmulo de lixo – como fatores de risco independentes para infecção pela *Leptospira*. Baixo nível sócio-econômico também foi associado com risco de infecção. Em um segundo estudo, nós comparamos a incidência de infecção pela *Leptospira*, determinada para uma coorte seguida por três anos no Pau da Lima, com as incidências de desfechos graves da leptospirose, determinadas para população de Salvador através de vigilância hospitalar no mesmo período. Nós identificamos que grupos etários mais velhos e sexo masculino estavam associados a maiores incidências de infecção pela *Leptospira*, leptospirose grave, hemorragia pulmonar maciça e mortalidade. Entretanto, o risco associado à idade adulta e ao sexo masculino foi maior para leptospirose grave (RR: 7,2 e 6,6, respectivamente) do que para infecção pela *Leptospira* (RR: 2,5 e 1,6, respectivamente), sugerindo que idade e sexo apresentam influência no risco de progressão para doença grave após a infecção. Entre os casos de leptospirose grave, idade acima de 45 anos foi fator de risco para óbito e sexo feminino foi fator de risco para hemorragia pulmonar maciça. Nossos achados sugerem que intervenções para controle da leptospirose em comunidades carentes devem ser direcionadas para criação de infra-estrutura de saneamento e redução da desigualdade social. Além disso, estudos para determinar os mecanismos patogênicos da influência da idade e do sexo na progressão para leptospirose grave após a infecção serão importantes para o desenvolvimento de vacinas ou tratamentos capazes de modular a progressão da infecção e prevenir formas graves de doença.

ABSTRACT

Severe leptospirosis has emerged as a significant urban health problem due to the worldwide expansion of slum settlements. Prevention measures are critical to reduce the morbidity and mortality associated with the disease. However, the lack of population-based information on determinants for *Leptospira* infection and on determinants for development of severe disease after infection has hampered the implementation of interventions. Our aims are to identify risk factors for *Leptospira* infection in an urban slum and to determine the influence of age and sex on the risk for infection and disease progression after infection. In a cross-sectional study conducted at Pau da Lima community in Salvador, we found that 15% of the 3,171 residents included in the study had serological evidence for prior *Leptospira* infection. Application of Geographical Information System and statistical modeling approaches identified sanitation deficiencies - open sewers, flooding-risk areas and refuse collections - as independent risk factors for *Leptospira* infection. Low socio-economic status was also associated with infection risk. In a second study, we compared the incidence of *Leptospira* infection, determined for a cohort followed for three years in Pau da Lima, with the incidences for severe outcomes of leptospirosis, determined for the Salvador population during hospital-based surveillance in the same period. We found that older age groups and male sex were associated with increased incidences of *Leptospira* infection, severe leptospirosis, severe pulmonary hemorrhage syndrome and mortality. However, the associated risk for adult age and male sex was greater for severe leptospirosis (relative risk [RR]: 7.2 and 6.6, respectively) than for *Leptospira* infection (RR: 2.5 and 1.6, respectively), suggesting that age and sex influence the risk for disease progression after infection. Among the cases of severe leptospirosis, age greater than 45 years old was risk factor for death and female sex was risk factor for severe pulmonary hemorrhage. Our findings suggest that interventions to control urban leptospirosis should address the creation sanitation infra-structure and reduction of social inequalities. In addition, studies to determine the pathogenic mechanisms for the age and sex influence on the progression to severe leptospirosis after infection will be significant for the development of vaccines or treatments which can modulate infection progression and prevent severe forms of the disease.

LISTA DE ABREVIATURAS

ArcGIS: Programa para análises de dados espaciais

CDC: Centro para Controle e Prevenção de Doenças, EUA

CI: Intervalo de confiança

CONDER: Companhia de Desenvolvimento Urbano do Estado da Bahia

ELISA: Ensaio de Imunoabsorbância Ligado a Enzima

Epi Info: Programa para análises de dados estatísticos

f(infection): Coeficiente de risco estimado por modelos aditivos generalizados

GAM: Modelos aditivos generalizados

GIS: Sistema de Informação Geográfica

IQR: Intervalo interquartil

MAT: Teste de microaglutinação

m: metros

m^2: metros quadrados

N: Número

NS: Não significante

PCR: Reação em cadeia da polimerase

PR: Razão de prevalência

R: Programa para análises de dados estatísticos

RR: Razão de risco

Real-time PCR: Reação em cadeia da polimerase em tempo real

SAS: Programa para análises de dados estatísticos

SD: Desvio padrão

SHPL: Síndrome de hemorragia pulmonar grave associada à leptospirose

SPHS: Síndrome de hemorragia pulmonar grave associada à leptospirose

SIG: Sistema de Informação Geográfica

UTI: Unidade de terapia intensiva

WHO: Organização Mundial de Saúde

WinBUGS: Programa para análises de dados estatísticos

SUMÁRIO

1. INTRODUÇÃO

A leptospirose, doença causada por espiroquetas do gênero *Leptospira*, é considerada a zoonose de maior distribuição mundial (WHO 1999; Levett 2001). As leptospiras são espiroquetas móveis, obrigatoriamente aeróbicas que dividem características de bactérias Gram-positivas e Gram-negativas. Até o presente, mais de 200 sorovares patogênicos de *Leptospira* foram descritos com base em diferenças genéticas e antigênicas entre os isolados (Faine et al. 1999; Levett 2001). O genoma da *Leptospira* consiste de dois cromossomos circulares e o seqüenciamento completo foi recentemente concluído para isolados dos sorovares Lai, Copenhageni e Hardjo (Ren et al. 2003; Nascimento et al. 2004; Bulach et al. 2006). O conhecimento do genoma da *Leptospira* e os avanços nas técnicas de manipulação genética permitiram a identificação da lipoproteína de superfície Loa22 como o primeiro fator de virulência associado à *Leptospira* (Ristow et al. 2007).

Diversas espécies de mamíferos servem de reservatórios para a *Leptospira* e mantém a transmissão da leptospirose na natureza. Como determinadas espécies de reservatórios costumam estar associadas a alguns sorovares, o conhecimento sobre quais são os reservatórios e os sorovares circulantes em uma região é essencial para o entendimento da epidemiologia da leptospirose no local (Levett 2001; Bharti et al. 2003). Uma vez infectados, as espécies de reservatórios apresentam colonização persistente dos túbulos proximais renais e disseminam de forma assintomática o organismo para o ambiente através da urina (Levett 2001; Bharti et al. 2003). Usualmente a transmissão entre reservatórios da mesma espécie ocorre por contato direto entre os animais e a prevalência da infecção aumenta com idade (Levett 2001). O sorovar que predomina no ambiente urbano é o Copenhageni e o seu reservatório principal é o *Rattus norvegicus* (rato marrom ou rato de esgoto) (Ko et al. 1999;

Pereira et al. 2000; Romero et al. 2003). A infecção dos humanos ocorre de forma acidental por contato direto ou indireto da pele não íntegra ou de superfícies mucosas com a urina de um animal infectado (Levett 2001).

O período de incubação médio após a infecção de um hospedeiro humano por leptospiras patogênicas é de 7 a 14 dias. A infecção é capaz de produzir uma grande variedade de manifestações clínicas que vão de uma infecção sub-clínica seguida de soroconversão, até a duas formas clínicas bem reconhecidas: a de uma doença febril aguda auto-limitada, e a de uma doença grave e potencialmente letal que pode se apresentar por qualquer combinação entre insuficiência renal aguda, icterícia, sangramentos e pneumonite (Levett 2001; Bharti et al. 2003; McBride et al. 2005). A forma grave que se manifesta por icterícia, insuficiência renal aguda e sangramento é conhecida como síndrome de Weil e tem letalidade >10%. A forma grave associada a pneumonite e sangramento pulmonar maciço é conhecida como síndrome de hemorragia pulmonar associada à leptospirose (SHPL) e apresenta letalidade >50% (Gouveia et al. 2008). Estima-se que 90-95% dos pacientes que apresentam manifestações clínicas da leptospirose desenvolvam a forma leve e auto-limitada da doença e que 5-10% evoluam para formas graves (Levett 2001; Bharti et al. 2003). Os mecanismos patogênicos que determinam a progressão para formas graves da doença ou para infecções sub-clínicas permanecem desconhecidos, mas devem estar relacionados a características de virulência da *Leptospira*, a dose de inóculo durante a infecção, a características da resposta imune do hospedeiro ou a uma interação entre estes fatores (Bharti et al. 2003; McBride et al. 2005).

O início da doença, tanto nas formas brandas e auto-limitadas quanto nas formas graves, costuma ser súbito. O paciente apresenta febre alta, algumas vezes acompanhada de calafrios, cefaléia, mialgia, anorexia, prostração, náuseas e vômitos (Bharti et al. 2003). Este

quadro inicial é de difícil diagnóstico e se confunde com doenças como dengue, influenza, gastrenterite e outras viroses (Levett 2001). Para 90-95% dos pacientes, este quadro se resolve espontaneamente em poucos dias e não deixa seqüelas. Entretanto, 5-10% dos pacientes pode evoluir para formas graves da doença, o que em geral ocorre ainda na primeira semana de sintomas. Os pacientes apresentam intensificação das dores musculares e da cefaléia e podem apresentar complicações graves como icterícia, insuficiência renal aguda (inicialmente não oligúrica e hipocalêmica, mas que pode progredir para necrose tubular aguda), arritmias, hemorragias (que são bem características quando acometem as conjuntivas), tosse seca ou produtiva, hemoptise e insuficiência respiratória (Levett 2001; Bharti et al. 2003; McBride et al. 2005).

Diversos estudos foram realizados para determinar fatores prognósticos associados à doença. Embora não exista um escore de gravidade, pacientes que apresentam alteração do estado mental, oligúria, hipercalemia, altos níveis de creatinina e uréia, arritmias, hipotensão e sobretudo hemorragia pulmonar e insuficiência respiratória tem um elevado risco de óbito e devem ser tratados hospitalizados e em unidade de terapia intensiva se necessário (Daher 1999; Ko et al. 1999; Lopes et al. 2001; Panaphut et al. 2002; Lopes et al. 2004; Abgueguen et al. 2008).

Embora a leptospirose tenha distribuição mundial, a grande maioria dos casos ocorre nos países tropicais e em desenvolvimento onde a doença se apresenta de forma endêmica (Levett 2001; Bharti et al. 2003). A maior incidência da doença ocorre durante as estações chuvosas e provavelmente se devem a surtos (Ko et al. 1999; Tassinari et al. 2008). A Organização Mundial de Saúde estima que mais de um milhão de casos de leptospirose ocorra por ano (WHO 1999). No Brasil, mais de 10.000 casos de leptospirose grave são notificados a

cada ano (McBride et al. 2005). Entretanto, a dificuldade para estabelecimento do diagnóstico da doença em pacientes que apresentam formas leves da doença e a inexistência de um teste diagnóstico rápido e acessível faz com que o impacto global da leptospirose seja subestimado e baseado principalmente nos pacientes com formas graves da doença (Pappas et al. 2008).

Mais de 80% dos casos de leptospirose grave são identificados na população masculina adulta (Everard et al. 1995; Ko et al. 1999; WHO 1999; Lopes et al. 2004; Tangkanakul et al. 2005; Jansen et al. 2007). Este achado tem sido justificado pelo dogma de que a leptospirose é uma doença ocupacional e esporádica, associada a profissões masculinas como agricultura, pecuária, mineração, manutenção de esgotos e serviços militares (Levett 2001; Bharti et al. 2003). Somente na última década a leptospirose ganhou atenção como um importante problema de saúde pública global. Este reconhecimento deveu-se à emergência da SHPL em todo o mundo (Trevejo et al. 1998; McBride et al. 2005; Gouveia et al. 2008), à identificação de surtos de leptospirose durante desastres (Campanella 1999; Sanders et al. 1999) e atividades de recreação e turismo (CDC 1998; CDC 2001; Morgan 2002; Sejvar et al. 2003), e às grandes epidemias, como a ocorrida na Tailândia no final da década de 1990, onde mais de 45.000 casos de leptospirose foram identificados (Tangkanakul et al. 2005).

Embora a população de maior risco seja tradicionalmente representada por agricultores de subsistência da zona rural (Faine et al. 1999; Levett 2001; Bharti et al. 2003), atualmente a leptospirose emerge como uma doença urbana que acomete os moradores pobres de favelas de países em desenvolvimento (McBride et al. 2005; Riley et al. 2007). A rápida urbanização e a crescente desigualdade social levaram a um dramático aumento da população das comunidades carentes urbanas, onde a falta de saneamento básico favorece a transmissão da leptospirose pelos ratos. A importância da leptospirose urbana como problema de saúde

pública deverá tornar-se ainda maior no futuro, pois as Nações Unidas estimam que nos próximos vinte e cinco anos a população de moradores de favelas dobrará de um para dois bilhões de pessoas (UN-HABITAT 2003).

O diagnóstico laboratorial da leptospirose pode ser feito por visualização direta, por isolamento do organismo, por testes sorológicos ou por métodos moleculares (Levett 2001). A visualização direta do organismo em amostras de sangue ou urina é feita com o uso da microscopia de campo escuro, entretanto, este método tem baixa sensibilidade e especificidade sendo pouco empregado. Visualização direta do microorganismo em tecidos é tradicionalmente realizada pela coloração com prata, embora imunohistoquímica tenha maior sensibilidade e especificidade. Sangue, urina, líqüor e outras amostras biológicas podem ser utilizadas para isolamento do organismo por cultura. A amostra deve ser colhida antes do início de antibióticos e cedo no curso da doença, uma vez que a chance de isolamento é maior nos primeiros dias de sintomas. Uma ou duas gotas de sangue devem ser inoculadas em meio semi-sólido contendo 5-fluorouracil e as culturas devem ser examinadas por microscopia de campo escuro uma vez por semana por até treze semanas.

Os testes sorológicos são os mais utilizados para o diagnóstico da leptospirose. A micro-aglutinação (MAT) é considerada o teste de referência e baseia-se na identificação por microscopia de campo escuro de aglutinação do soro do paciente com antígenos vivos de diferentes sorogrupos da *Leptospira*. O teste é complexo e tem baixa sensibilidade com uso de soro agudo, sendo necessária uma amostra convalescente para avaliar se houve soroconvesão ou aumento de títulos entre as duas amostras. Outros testes sorológicos comumente utilizados incluem hemaglutinação indireta e ELISA (Enzyme Linked Immuno Sorbent Assay). Técnicas de biologia molecular como PCR (polymerase chain reaction**)** e Real-time PCR

(PCR em tempo real) vêm sendo empregadas como ferramentas de pesquisa e deve demorar até que os custos permitam o seu emprego rotineiro.

O tratamento da leptospirose é baseado no uso de antibióticos e de terapias de suporte (Levett 2001; Bharti et al. 2003; McBride et al. 2005). Embora haja controvérsia do benefício da antibioticoterapia na redução da letalidade associada à doença grave (Guidugli et al. 2000), o emprego de antibióticos parece reduzir a duração da febre, o tempo para normalização da função renal e o tempo de hospitalização (McClain et al. 1984 ; Watt et al. 1988). Penicilina cristalina ou ceftriaxone, e doxiciclina são os antibióticos recomendados para os pacientes hospitalizados e ambulatoriais, respectivamente (Levett 2001; Bharti et al. 2003; McBride et al. 2005). Seu uso deve ser iniciado tão cedo quanto possível após o diagnóstico clínico e epidemiológico. Medidas de suporte devem ser empregadas de acordo com a indicação clínica e incluem hidratação vigorosa, monitorização em unidade de terapia intensiva (UTI), uso de drogas vasoativas, ventilação mecânica e diálise (McBride et al. 2005). Experiência bem sucedida na UTI do Hospital Emílio Ribas, São Paulo, sugere que o início precoce e diário de hemodiálise em vez de tardio e em dias alternados, em conjunto com melhorias no cuidado do paciente que incluíram admissão precoce em UTI, uso de ventilação mecânica protetora e fisioterapia respiratória, foi capaz de reduzir a letalidade de pacientes com SHPL de 67% para 17% (Andrade et al. 2007).

A prevenção da leptospirose pode ser feita por medidas individuais e coletivas. Medidas individuais envolvem evitar ou reduzir a exposição a águas e solos potencialmente contaminados por leptospiras, assim com evitar o contato com animais potencialmente contaminados. Quando não for possível evitar o contato, o mesmo deve ser feito com luvas e botas. Evidências sugerem que uso de quimioprofilaxia com doxiciclina antes ou após

exposição é capaz de reduzir a taxa de ataque da doença, mas não impede a ocorrência da infecção sorológica (Takafuji et al. 1984 ; Sehgal et al. 2000). Portanto, seu uso deve ser considerado antes e após exposições de alto risco para infecção (Bharti et al. 2003). Embora Cuba e China tenham desenvolvido vacinas para uso humano, estas vacinas têm proteção de curta duração e não geram proteção cruzada contra sorogrupos que não constam na vacina. Tais preocupações fizeram com que estas vacinas não fossem licenciadas fora de seus países (McBride et al. 2005). Medidas de prevenção coletivas, como desratização, são utilizadas com freqüência, entretanto, são custosas e precisam ser repetidas rotineiramente para ter benefício.

2. JUSTIFICATIVA

Nas últimas décadas a leptospirose emergiu como uma doença de moradores de comunidades carentes urbanas. A rápida migração populacional da zona rural para a zona urbana e a desigualdade social levaram ao crescimento desorganizado de comunidades pobres nas periferias de todas as grandes cidades brasileiras. Nestas comunidades, as deficiências sanitárias e a pobreza propiciam a transmissão da *Leptospira* por roedores. As Nações Unidas estima que nos próximos vinte e cinco anos a população mundial de moradores de favelas dobrará de um para dois bilhões de pessoas o que deverá aumentar a importância da leptospirose como um problema de saúde pública urbana.

Em Salvador, estudos sobre a epidemiologia da leptospirose urbana vêm sendo conduzidos desde 1996 e contribuíram substancialmente para identificar um novo padrão epidemiológico de epidemias cíclicas associadas a fortes chuvas. As principais características destas epidemias são: 1) ocorrência de surtos anuais acometendo as mesmas comunidades carentes durante os meses de chuva e associados a desfechos clínicos graves (Ko et al. 1999; Flannery et al. 2001; Sarkar et al. 2002); 2) ocorrência de transmissão no ambiente peri-domiciliar, influenciada por fatores ambientais e pela infestação por roedores (Sarkar et al. 2002; Maciel et al. 2008); e 3) o agente causador dos surtos é um único sorovar, *L. interrogans* sorovar Copenhageni, que é associado com ratos de esgoto como reservatório (Ko et al. 1999; Barocchi et al. 2001; Tucunduva de Faria et al. 2007).

Medidas de prevenção da leptospirose são necessidades urgentes. Entretanto, na conjuntura de escassez de recursos governamentais para grandes obras de urbanização e infra-estrutura, a identificação de áreas de maior risco para transmissão da bactéria e a

determinação de fatores de risco para a infecção são essenciais para guiar intervenções comunitárias de prevenção. Nós propomos um estudo para identificar deficiências de saneamento na comunidade que atuem como fatores de risco para infecção pela *Leptospira*.

Além disso, não está claro porque a maioria das pessoas expostas a leptospiras evoluem com infecções sub-clínicas enquanto uma pequena fração desenvolve formas graves da doença. Como mais de 80% dos casos de leptospirose grave ocorre entre homens adultos é possível que fatores relacionados ao sexo e à idade, como níveis hormonais e maturidade do sistema imune influenciem o risco de progressão da doença após a infecção. Esta hipótese tem sido pouco aventada porque tradicionalmente a leptospirose é considerada uma doença ocupacional. Entretanto, estudos de soroprevalência identificaram que homens e mulheres são infectados por leptospiras na mesma freqüência e que infecção em crianças é comum (Raoult et al. 1985; Johnson et al. 2004). Além disso, um estudo na Alemanha observou que homens foram hospitalizados por leptospirose mais freqüentemente que mulheres e tiveram maior risco de evoluir com sintomas graves como icterícia, insuficiência renal e sangramentos (Jansen et al. 2007). Os autores concluíram que a predominância de leptospirose no sexo masculino se deve a uma maior incidência de leptospirose grave nos homens e não a diferenças na incidência de infecção entre os sexos.

A definição do papel do hospedeiro na história natural da leptospirose será relevante para guiar estratégias de prevenção e para o desenvolvimento de vacinas ou tratamentos que visem modular a progressão da infecção para formas graves da doença. Nós pretendemos determinar a influência da idade e do sexo no risco de infecção e de desenvolvimento de formas graves da leptospirose ao comparar as incidências de infecção, leptospirose grave, SHPL e óbito, de acordo com idade e sexo.

3. OBJETIVOS

Geral

Identificar fatores de risco para infecção e para progressão para formas graves da doença a fim de guiar intervenções de prevenção na comunidade.

Específicos

1.1 Determinar a prevalência de infecção prévia pela *Leptospira*, definida pela presença de anticorpos aglutinantes contra a *Leptospira*, em moradores de uma comunidade de Salvador;

1.2 Determinar fatores de risco ambientais e sociais para a presença de anticorpos aglutinantes contra a *Leptospira* em moradores de uma comunidade de Salvador;

1.3 Determinar a influência da idade e do sexo sobre as incidências de infecção pela *Leptospira*, de leptospirose grave, da SHPL e da mortalidade por leptospirose;

1.4 Estimar o risco de desenvolvimento de leptospirose grave após infecção pela *Leptospira* e o risco de progressão para SHPL e óbito após desenvolvimento de leptospirose grave, de acordo com a idade e o sexo.

4. MANUSCRITO 1

TÍTULO:

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].

Publicado no PLoS Neglected Tropical Diseases, em 23 de Abril de 2008.

RESUMO:

A leptospirose, uma zoonose potencialmente letal, tornou-se um importante problema de saúde urbana em conseqüência da expansão de comunidades (favelas) com condições ambientais favoráveis para a transmissão da doença pelos ratos. Neste estudo de prevalência com mais de 3.000 moradores de uma comunidade de Salvador, Brasil, a aplicação de Sistema de Informação Geográfica (SIG) e de modelagem estatística permitiu identificar deficiências específicas na infra-estrutura de saneamento da comunidade - esgotos abertos, acúmulo de lixo, sistema de drenagem de água inadequado - que servem como fontes de transmissão da *Leptospira*. Além dos atributos ambientais da comunidade, baixo nível socioeconômico foi identificado como fator de risco independente para infecção. Estes resultados indicam medidas de prevenção da leptospirose deverão ser direcionadas para melhorar o saneamento básico e para reduzir as diferenças sociais responsáveis por desfechos de saúde desiguais entre moradores de uma mesma comunidade.

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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.

Citation: 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 Negl Trop Dis 2(4): e228. doi:10.1371/journal.pntd.0000228

Editor: Ricardo E. Gurtler, Universidad de Buenos Aires, Argentina

Received January 22, 2008; **Accepted** March 27, 2008; **Published** April 23, 2008

Funding: This work was supported by the US National Institutes of Health (grants NIAID R01 AI052473, FIC D43 TW00919), Brazilian National Research Council (grants 300861/1996, 473082/2004, 420067/2005, 305723/2006, 150176/2007), and Oswaldo Cruz Foundation (grant 0250.250.102). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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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.
doi:10.1371/journal.pntd.0000228.g001

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

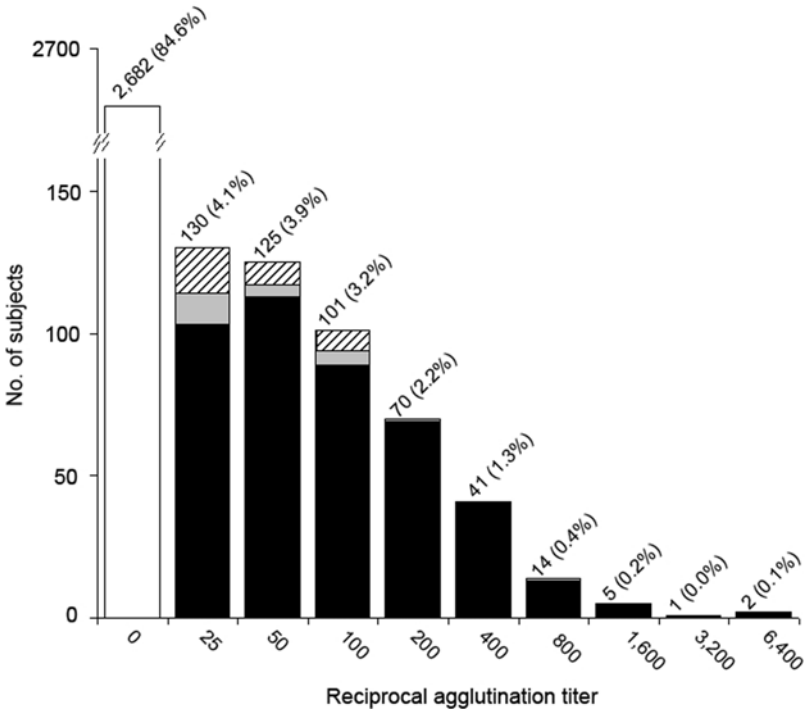

Figure 2. Distribution of reciprocal microscopic agglutination test titers for 3,171 subjects from the slum community site. Labels above the bars indicate the number of subjects (% of total), according to their highest reciprocal titer. The open bar represents seronegative subjects. Subjects with highest 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.
doi:10.1371/journal.pntd.0000228.g002

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.
doi:10.1371/journal.pntd.0000228.t001

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).

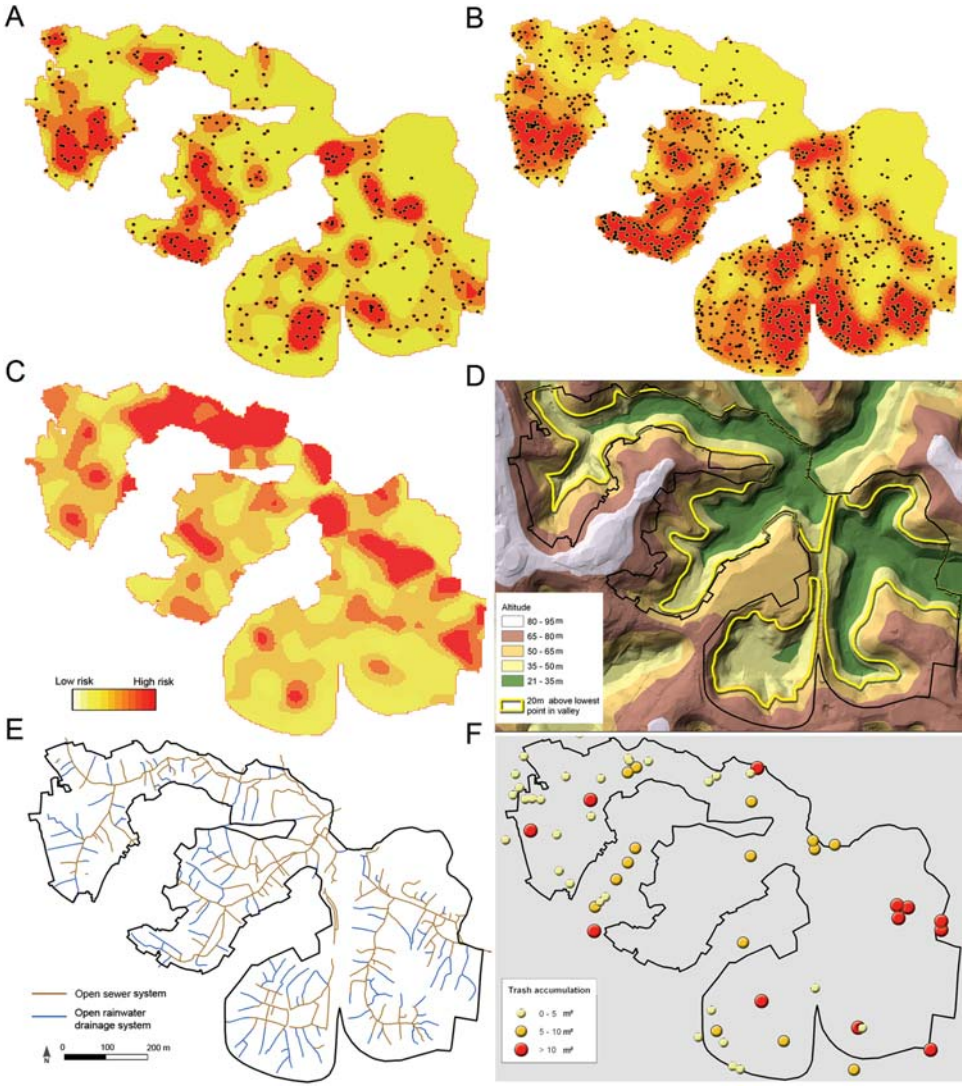

Figure 3. Spatial distribution of subjects with *Leptospira* antibodies and all enrolled subjects, according to place of residence, and environmental attributes of the community site. *Panels A* and *B* show the smoothed Kernel density distribution of subjects with *Leptospira* antibodies (N = 489) and 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).
doi:10.1371/journal.pntd.0000228.g003

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

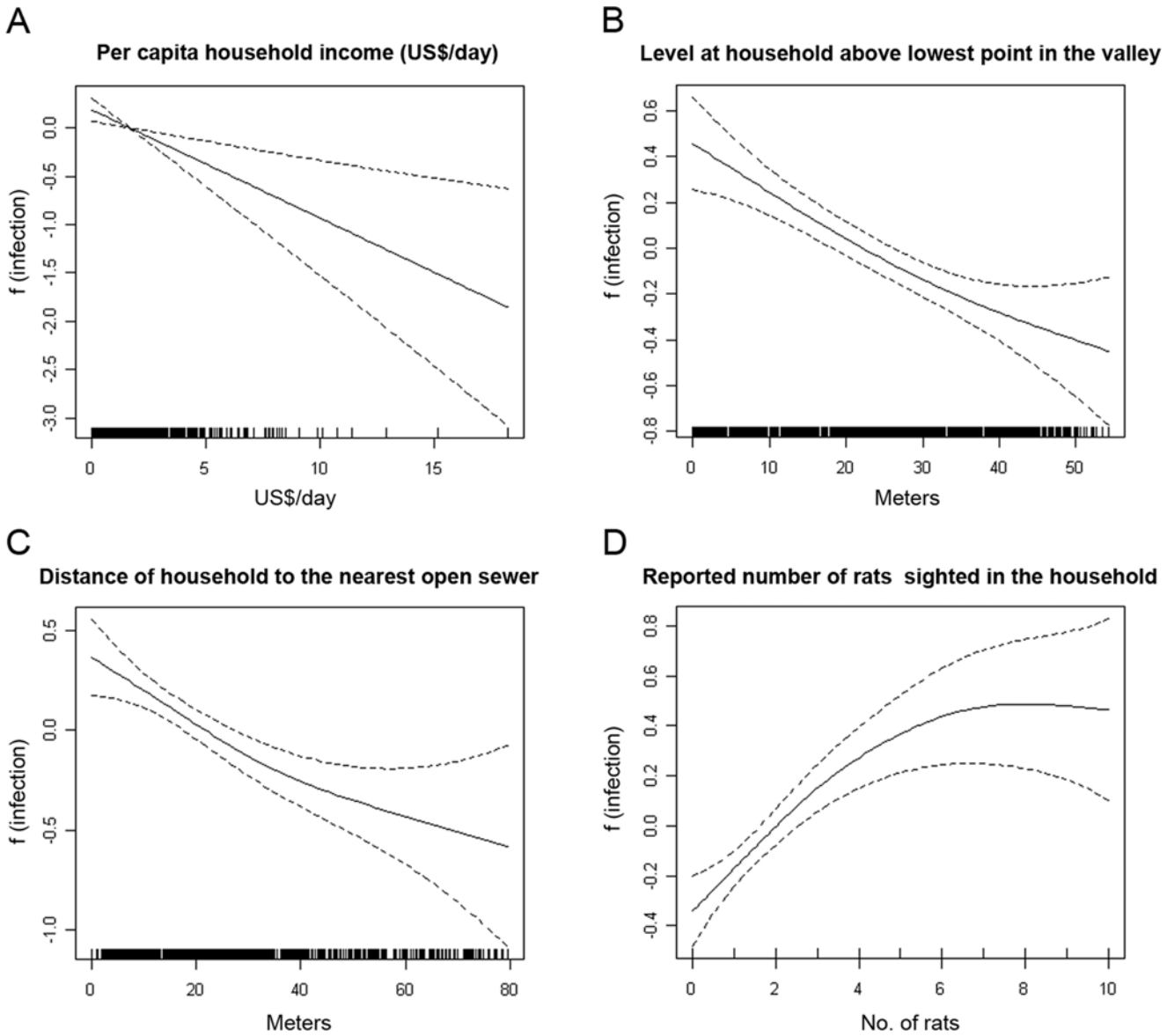

Figure 4. Generalized additive models (GAM) of the association between the risk of acquiring *Leptospira* antibodies and continuous variables of (*A*) per capita daily household income, (*B*) level of household in meters above the lowest point in valley, and (*C*) distance in meters to the nearest open 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.
doi:10.1371/journal.pntd.0000228.g004

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 $\geq$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)

Acknowledgments

We would like to thank team members from the Urban Health Council of Pau da Lima and Oswaldo Cruz Foundation, Rosan Barbosa, Reinaldo Barreto, Jorge Costa, Maria Raimunda da Cruz, Ana Carla Duarte, Leila Gouveia, Analéa Lima, Simone Nascimento, Osmar Paixão, Amaro Silva and Érika Sousa who participated in the data collection for the study. We would also like to thank the Company for Urban Development of the State of Bahia (CONDER) for providing digital maps of the study site, Elves Maciel and Alicia Chang for their assistance in designing the study; Claudio Pereira da Sá (*in memorium*) and Edilane Gouveia for assistance with the statistical analysis; Earl Francis Cook Jr. for critical advice during data analysis and manuscript preparation; and Lee Riley, Art Reingold and Maurício Barreto for reviewing the final manuscript. Finally, this work could not be accomplished without the joint collaborative effort of the resident associations that comprise the Urban Health Council of Pau da Lima and community leaders and residents.

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.

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5. MANUSCRITO 2

TÍTULO:

Influence of Age and Sex on *Leptospira* Infection, Disease Progression after Infection and Severe Outcomes from Leptospirosis.

[Influência da Idade e do Sexo na Infecção pela *Leptospira*, na Progressão da Doença após a Infecção e nos Desfechos Graves da Leptospirose].

A ser submetido.

RESUMO:

Mais de 80% dos casos de leptospirose grave ocorrem em homens adultos, portanto fatores associados ao sexo e à idade devem influenciar o risco de infecção por *Leptospira* ou o risco de progressão da doença após a infecção ou ambos. Nós determinamos o papel do sexo e da idade sobre estes riscos através da comparação das incidências estratificadas por sexo e idade de: infecção pela *Leptospira* – determinada em uma coorte seguida por três anos na comunidade de Pau da Lima, Salvador-BA – leptospirose grave, hemorragia pulmonar maciça por leptospirose e mortalidade – determinados para a cidade de Salvador no mesmo período. Nós observamos que as incidências de infecção pela *Leptospira*, leptospirose grave, hemorragia pulmonar maciça e mortalidade foram maiores para os homens e para os grupos etários mais velhos. Entretanto, a indivíduos do sexo masculino e com mais de 15 anos tiveram maior risco de leptospirose grave (RR: 7,2 e 6,6, respectivamente) do que de infecção pela *Leptospira* (RR: 2,5 e 1,6, respectivamente), sugerindo que idade e sexo influenciam o risco de progressão para doença grave após a infecção. Entre os casos de leptospirose grave, idade acima de 45 anos foi fator de risco para óbito e sexo feminino foi fator de risco para hemorragia pulmonar maciça. Estudos para determinar os mecanismos patogênicos da influência da idade e do sexo na progressão para leptospirose grave após a infecção poderão ajudar no desenvolvimento de vacinas ou imunomoduladores capazes de intervir na progressão da infecção e prevenir formas graves de doença.

Influence of age and sex on *Leptospira* infection, disease progression after infection and severe outcomes from leptospirosis

Running Title: Role for age and sex on outcomes from leptospirosis

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Abstract

Background. The larger burden of severe leptospirosis among males and adults is thought to be related to an increased risk for *Leptospira* infection in this population. Whether male adults also have increased risk for development of severe leptospirosis after infection is not clear. We conducted an observational study to investigate the influence of age and sex on the risk for *Leptospira* infection, severe leptospirosis and severe outcomes of the disease.

Methods. In Salvador, Brazil, we measured annual incidence of *Leptospira* infection during three years of follow up of a community-based cohort. We measured annual incidence of severe leptospirosis, leptospirosis-associated severe pulmonary hemorrhage syndrome (SPHS) and leptospirosis mortality during three years of hospital-based surveillance for severe leptospirosis. We used the microagglutination test (MAT) to identify cases of *Leptospira* infection, and MAT and ELISA to confirm suspected cases of severe leptospirosis. Incidences were stratified by age and sex and used to estimate age and sex-specific risks for progression to severe leptospirosis after *Leptospira* infection. We used logistic regression analysis to determine the age and sex-specific risks for death and SPHS among the cases of severe leptospirosis.

Results. Age increase and male sex were associated with higher incidences of *Leptospira* infection, severe leptospirosis, SPHS and mortality. In comparison to children with 5-14 years of age, subjects ≥15 years had 2.49 (95% CI: 1.53-4.07) and 7.15 (95% CI: 3.92-13.03) greater risk for *Leptospira* infection and severe leptospirosis, respectively. In comparison to females, males had 1.55 (95% CI: 1.12-2.14), and 6.64 (95% CI: 4.92-8.96) greater risk for *Leptospira* infection and severe leptospirosis, respectively. Males had a fourfold greater risk for progression to severe leptospirosis after infection in comparison to females. Among the patients with severe leptospirosis, age increase was associated with death (*P* value for trend <

.05), but not with SPHS. In contrast, female sex increased the risk of SPHS in 2.49 (95% CI: 1.05-5.75) times, but had no influence on the risk of death.

Conclusions. Older age and male sex positively influence the risk for *Leptospira* infection and development of severe leptospirosis after infection. Age also influences the risk of death among cases of severe disease. In contrast, female sex increases the risk for SPHS. Studies to determine the pathogenic mechanisms behind the influences of age and sex on the *Leptospira* infection progression are warranted.

Key words: Leptospirosis, age, sex, incidence, mortality, pulmonary hemorrhage syndrome

INTRODUCTION

Leptospirosis is a zoonotic disease transmitted to humans by exposure to environment contaminated by urine of reservoirs [1]. Individuals who are infected can develop a wide spectrum of clinical manifestations, ranging from asymptomatic infections to severe icterohaemorrhagic presentations, such as Weil's disease and severe pulmonary hemorrhage syndrome (SPHS), for which case fatality rates are greater than 10% and 50%, respectively [1, 2]. It is not known why certain exposed individuals develop asymptomatic or mild infections while others progress to develop severe disease, but it may be related to age and sex factors since population-based studies have shown that severe disease is uncommon among children and that 80% of the cases of severe leptospirosis occurs among males [3-7].

Traditionally, the disproportional increased incidence of severe leptospirosis among adult males has been explained by the dogma that leptospirosis is a disease of occupational transmission, typically affecting the male adults who works as miners, fishers, farmers or in military or sewer services [1]. However, leptospirosis epidemics have been increasingly recognized in association with environmental exposures that are not primarily associated with age and sex, such as during extreme rainfall events [8-10], weather-related disasters [11, 12] and living in conditions of inadequate sanitation [13, 14]. In addition, seroprevalence studies have identified that presence of antibodies against *Leptospira* is common among children [15, 16] and occurs in similar frequency between males and females [15, 17]. An alternative explanation is that age and sex-related factors influence the risk for progression to severe leptospirosis after infection acquisition. This hypothesis is supported by studies about the severity of the disease that found that older age is associated with higher case fatality rate [5, 6] and that male sex increases the risk for jaundice, renal failure, hemorrhage and hospitalization [4].

In Salvador, Brazil, we were able to estimate the age and sex-specific incidences of *Leptospira* infection from a community-based cohort study, and the age and sex-specific incidences of severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality from a city-wide surveillance for severe leptospirosis. As a result, we had a unique opportunity to investigate the influence of age and sex on the risk for *Leptospira* infection and progression to severe outcomes after infection.

METHODS

Community-based cohort study.

During three years, we conducted a cohort study in Pau da Lima, a community situated in the periphery of Salvador, Brazil. The study site has been previously described [16]. Briefly, 85% of its population were squatters who did not have legal title to their domiciles. The median household per capita income was US$ 1.30 per day.

Between February 2003 and May 2004, the study team conducted a census within the study site and identified 14,122 inhabitants living in 3,689 households. We used a computer-based random number generator to select a list of 684 (19%) households from a database of all enumerated households within the study site. All subjects who slept three or more nights per week in a sampled household and had five or more years of age were eligible to be a cohort member. Among 2,419 subjects who were eligible to be a cohort-member, 2,003 (83%) agreed to participate and were enrolled in the study according to written informed consent procedures approved by the Fundação Oswaldo Cruz, Brazilian Ministry of Health and Weill Medical College of Cornell University.

During cohort enrollment the study team 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 in the household and workplace [16]. At enrolment and once a year during three consecutive years, the study team visited the household of the study participants in the post-rainy season (October to January in the 1st year of follow up and November to February in the 2nd and 3rd years of follow up) to collect sera samples that were used to identify cases of *Leptospira* infection.

Serologic evaluation for* Leptospira *infection

We used the microagglutination test (MAT) to evaluate subjects' sera for the presence of agglutinating antibodies against *Leptospira*. The MAT panel included five of the reference strains recommended by the World Health Organization (Royal Tropical Institute, the Netherlands), which included *Leptospira interrogans* serovars Autumnalis, Canicola and Copenhageni, *L. borgspetersenii* serovar Ballum, *L. kirschneri* serovar Grippotyphosa, and one clinical isolate, *L. interrogans* serovar Copenhageni strain Fiocruz L1–130 [5]. The use of this panel had the same performance in identifying MAT-confirmed cases of severe leptospirosis during surveillance in Salvador than did the extended battery recommended by the WHO [5]. 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. Positive and negative control sera were always included in the battery. A case of *Leptospira* infection was defined as a cohort subject who seroconverted from a MAT titer of zero to a follow-up titer ≥1:50 or who presented a fourfold rise in the MAT titer between two consecutive evaluations.

Hospital-based surveillance for leptospirosis. Active surveillance for severe leptospirosis has been conducted in Salvador, Brazil (population 2.4 million inhabitants) since 1996. We included in this study leptospirosis cases identified during the three-year period between January 1, 2003 and December 31, 2005. Cases were identified at Hospital Couto Maia, the state infectious diseases hospital, which is the reference center for leptospirosis in Salvador. Notification of leptospirosis cases is mandatory in Brazil and this hospital reports >95% of the cases from the city. The study team consecutively identified case-patients who were admitted to this hospital and met the clinical definition for severe leptospirosis, which included characteristic late phase manifestations of leptospirosis (jaundice and serum aminotransferase activities less than 20 times the normal upper limit [30 U/L]; oliguria, creatinine ≥1.5 mg/dl or urea ≥75 mg/dl; or hemorrhagic diathesis) in a patient with an acute febrile disease [5]. Patients who fulfilled the case definition were enrolled into the study according to informed consent protocols approved by the Fundação Oswaldo Cruz, Brazilian Ministry of Health and Weill Medical College of Cornell University. We used standardized forms to interview the patients or their family members and to extract information from medical charts on clinical presentation, laboratorial exams and outcome.

Laboratory confirmation of leptospirosis

Laboratory confirmation of the cases were performed using blood samples collected during hospital admission, on day 4 or 5 of hospitalization, and 2 weeks after the collection of the first sample. A laboratory-confirmed case of leptospirosis was defined as a case who presented a fourfold rise in the MAT titer between paired samples, a single titer ≥1:800 [5], or a positive result on the immunoglobulin M (IgM) ELISA (Bio-Manguinhos, Rio de Janeiro, Brazil) [19]. The same panel of five reference strains (WHO Collaborative Laboratory for Leptospirosis, Royal Tropical Institute, Holland) and one clinical isolate [5] used in the cohort

study was used in the surveillance for severe leptospirosis. Positive and negative control sera were always included in the panel.

Statistical analysis.

Data from both the cohort study and from the hospital-based surveillance were double-entered and validated using an Epi Info 3.3.2 software system (Centers for Disease Control and Prevention, USA) database. Epi Info 3.4.3 and SAS 9.1 were used for data analysis. Mean annual cumulative incidence of *Leptospira* infection was calculated for the cohort subjects who completed the three years of follow up. Mean annual cumulative incidences of severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality were calculated for the population of Salvador using the cases identified during the three-year surveillance period. Incidences were stratified by age and sex and presented as the number of cases per 1,000 or 100,000 population, with respective 95% confidence intervals. For this study, we defined subjects with 5-14 years of age and subjects with ≥15 years of age as children and adults, respectively. The Salvador population count from the 2000 national census was used for incidence calculations [20].

The chi square test was used to evaluate whether there were annual variations among the incidences measured in each of the three years of study. The chi square test for linear trend was used to determine whether there was linear association between age and the incidences of *Leptospira* infection, severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality. Risk ratios and 95% confidence intervals were used to evaluate whether adults and males were associated with *Leptospira* infection and severe leptospirosis.

We used the mean annual incidence of *Leptospira* infection for the Pau da Lima cohort to project the number of cases of *Leptospira* infection in the city of Salvador. We used the projected number of cases of *Leptospira* infections and the ascertained number of cases of severe leptospirosis for the period between 2003 and 2005 in Salvador to estimate the risk ratio and 95% confidence interval for development of severe leptospirosis after infection, according to age and sex. We used logistic regression analysis to estimate the influence of age and sex as independent risk factors for progression to SPHS and death among the cases of severe leptospirosis identified during the study.

RESULTS

Among the 2,003 cohort-members, 1,300 (65%) subjects completed the three years of follow-up. The mean annual dropout rate was 13.4%. Reasons to lose the follow-up included migration (469 cases; 67%), inability to find the participant (109; 16%), refusal o participate (104; 15%) and death (14; 2%). Subjects who were lost-to-follow-up had higher proportions of males than subjects who completed follow up (49 vs. 42%, P<0.01) but were not different with respect to age, socioeconomic level and exposure to environmental risks. We identified 140 infections among the 1,300 subjects who completed follow-up. For 93% of the infections the highest MAT titer was against serovar Copenhageni. The mean annual incidence of *Leptospira* infection was 35.9 (95% confidence interval [CI]: 30.3-42.2) per 1,000 population (Table 1). Incidences of infection in 1st, 2nd and 3rd year of follow up did not differ significantly (40.8, 33.8 and 33.1 per 1,000 persons, respectively; $P > .05$). Among 322 and 71 subjects who completed only one and two follow-up years, respectively, the annual incidence of infection was 40.8 and 14.1 per 1,000 population, respectively. These rates did not differ significantly from those for the closed cohort ($P < .05$).

During the three-year period between January, 2003 and December 2005, the surveillance identified 341 cases of severe leptospirosis in Salvador. Laboratorial confirmation of the diagnosis was made for 281 (82%) of the cases. For the 252 cases that were confirmed by MAT, 97% had the highest MAT titer against the serovar Copenhageni. Confirmed and unconfirmed cases had similar clinical presentation, except that unconfirmed cases were older and had more severe outcomes, such as SPHS and death (Supplemental table 1). Unavailability of paired blood sample was the main reason for non-confirmation of cases (Supplemental table 1). Mean annual incidence of severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality for Salvador were 5.1, 0.4 and 0.9 per 100,000 population, respectively (Table 1). Annual incidence of severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality were lower in 2003 and 2004 in comparison to 2005 (4.3 and 4.0 vs. 5.7 per 100,000 population for severe leptospirosis, $P < .05$; 0.3 and 0.2 vs. 0.8 per 100,000 population for SPHS, $P < .05$; and 0.6 and 0.7 vs. 1.1 per 100,000 population for mortality, $P > .05$, respectively).

Incidences of *Leptospira* infection, severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality were found to have linear trend associations with age (*P* value for linear trend < .05 for all incidences; Figure 1). Incidence of *Leptospira* infection was high for all age groups, including children, and peaked among young adults (25-34 years of age) and adults with ≥55 years of age (Table 1, Figure 1A). In contrast, incidence of severe leptospirosis was very low among children and peaked among adults 45-54 years old (Table 1, Figure 1B).

Adults had increased risk for *Leptospira* infection (RR: 2.49; 95% CI: 1.53-4.07), and severe leptospirosis (RR: 7.15; 95% CI: 3.92-13.03) in comparison to children. The incidence of

SPHS and death for children was virtually null, since none case of SPHS and death was identified among children. In addition, the male population had increased risk for *Leptospira* infection (RR: 1.55; 95% CI: 1.12-2.14), severe leptospirosis (RR: 6.64; 95% CI: 4.92-8.96), leptospirosis-associated SPHS (RR: 2.92; 95% CI: 1.35-6.30) and leptospirosis mortality (RR: 4.04; 95% CI: 2.18-7.47) in comparison to the female population. Of note, adult age and male sex had much stronger associations with severe leptospirosis than with *Leptospira* infection. Thus, the burden of severe leptospirosis among male adults appears to be more influenced by age and sex-specific differences in the risk for progression to severe leptospirosis after infection than by differences in the infection risk.

Assuming that the age and sex-specific incidences of *Leptospira* infection found during the cohort study were the same for the population of Salvador, we estimated the risk for progression to severe leptospirosis after *Leptospira* infection (Table 2). The overall risk for progression to severe leptospirosis after infection was low (1.4 cases of severe disease per 1,000 infections). The age group 45-54 years of age had the higher risk for progression to severe leptospirosis after infection and, among males, children had the lowest risk. The male population had an estimated risk for development of severe leptospirosis after infection four times greater than the female population (2.1 and 0.5 cases of severe disease per 1,000 infections, respectively). The male increased risk for progression to severe leptospirosis after infection was identified for all age groups except than for children.

Univariate analysis for the cases of severe leptospirosis identified an association between age and case fatality rate. While none of the children 5-14 years old evolved to death, 10%, 14%, 15%, 27% and 37% of the patients with 15-24, 25-34, 35-44, 45-54 and ≥55 years old died during hospitalization, respectively (*P* value for linear trend < .05; Figure 2A). Although case

fatality rate was higher among women than among men, the difference was not statistically significant (27% vs. 16%, RR: 1.65; 95% CI: 0.97-2.82). Logistic regression analysis with adjustment for age and sex confirmed a positive association between age increase and death (Table 3).

In contrast, univariate analysis found that age was not associated with SPHS (*P* value for linear trend < .05; Figure 2B), and that female sex was a risk factor for SPHS. While 18% of the women with severe leptospirosis developed SPHS during hospitalization, only 8% of the men presented this outcome (RR: 2.28, 95% CI: 1.12-4.63). Logistic regression analysis with adjustment for age and sex confirmed the association between female sex and SPHS (Table 3).

DISCUSSION

Key questions on the epidemiology of leptospirosis concerns why only a fraction of the infected persons develop severe disease after infection and why the majority of cases of severe leptospirosis occur among male adults. In this investigation, we combined data from a cohort study for *Leptospira* infection and from a hospital-based surveillance for severe leptospirosis to simultaneously determine age and sex-specific incidences of *Leptospira* infection, severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality. Our findings indicate that age and sex have significant influences on the risks for *Leptospira* infection and progression to severe outcomes from leptospirosis after infection.

Traditionally, the dogma that leptospirosis is an occupational disease that place adult males at increased risk for infection has justified why severe leptospirosis is an uncommon disease among children and women. However, our results suggest that this concept may be only part

of the truth. We found that adults and males do have higher risks for *Leptospira* infection, but, in addition, we provided evidences to support that age increase and male sex are also at increased risk for developing severe leptospirosis after infection. The evidences for an association between age increase and risk for progression to severe disease were: (1) adults had 2.5 and 7.2 times greater risk for *Leptospira* infection and severe leptospirosis, respectively, in comparison to children, suggesting that older age has a larger impact on the risk for progression to severe disease than on the risk for infection; (2) the age group of 45-54 years of age had the highest estimated risk for progression to severe leptospirosis after infection, while, among males, children with 5-14 years of age had the lowest risk; and (3) age increase was associated with higher case fatality rate. In addition, none of the chidren evolved to death. The evidences for an association between male sex and risk for progression to severe disease were: (1) men had 1.6 and 6.6 times greater risk for *Leptospira* infection and severe leptospirosis, respectively, suggesting that male sex has a larger impact on the risk for progression to severe disease than on the risk for infection; and (2) males had an estimated risk for progression to severe leptospirosis after infection four times greater than the females.

Several studies have presented results that support the hypothesis that age influences the risk for progression to severe disease after infection. Children with severe leptospirosis has been shown to have a better prognosis than adults [3, 6], which indicates that progression to severe outcomes is less common among children. Older age has also been shown to be an independently associated with risk for death [5]. In addition, evidence that women have a milder leptospirosis has been published. Jansen et al. (2004) evaluated 338 patients with laboratory-confirmed leptospirosis reported in Germany during 1997–2005 and identified that male patients were more likely than female patients to be hospitalized and exhibit severe symptoms, such as jaundice, renal dysfunction and hemorrhages [4]. They concluded that

"reports on male predominance in leptospirosis may thus reflect sex-related variability in the incidence of severe disease, rather than different infection rates".

An interesting finding of our study was that although women had a lower risk for progression to severe disease, they had a higher risk for the development of SPHS after adjusting for age. An association between female sex and SPHS has been shown previously in Salvador, but the leptospirosis casuistic was similar than that included in this study [21]. Epidemiological investigations in other settings are necessary to confirm this finding.

The pathogenic mechanisms through which age and sex influence the risk for progression to severe disease and severe outcomes after infection remains to be elucidate. It is possible that different behaviors between children and adults and between males and females determine the intensity of exposure to a contaminated environment and consequently the inoculum dose of *Leptospira* during infection. As an alternative hypothesis, differences in host-specific immune response after *Leptospira* infection may be driven by age and sex-related factors, such as immune system maturity and hormone levels. In addition, repetitive *Leptospira* infections and prior acquisition of antibodies against *Leptospira* are potentially influenced by age and sex and may have a role on the risk for progression to severe disease after infection.

Our investigation has strengths and limitations. This is the first study to simultaneously estimate the incidence of *Leptospira* infection, severe leptospirosis, leptospirosis-associated SPHS and leptospirosis mortality for a large urban population. Since it is not practical to determine all cases of *Leptospira* infection for a large city as Salvador, we used the incidence of *Leptospira* infection from the Pau da Lima cohort as a proxy for the incidence of

Leptospira infection for Salvador. This approach enabled us to estimate the age and sex-specific risk ratios for progression to severe disease after infection.

As a limitation, we assumed that the cohort population from the Pau da Lima community represents the population of Salvador. Although this assumption is reasonable, since 67% of the population of Salvador lives in similar social and environmental conditions than the inhabitants of Pau da Lima, it is not completely correct. Pau da Lima is a poor community with high incidence of severe leptospirosis. Thus, it is possible that the *Leptospira* infection incidences measured for the Pau da Lima cohort were higher than the real *Leptospira* infection incidence for Salvador. This inaccuracy may have lead to an underestimation of the risk for progression to severe disease after infection. In addition, bias may have resulted from eligible participants refusal to be enrolled into the study and from loss-to-follow-up of cohort-members. However, we compared incidences of *Leptospira* infection between those who completed and those who did not complete follow up but were followed for at least one year and we found similar incidences between them. Finally, other variables, such as days of symptoms, could have confounded the association between age and death, and between sex and SPHS. The inclusion of days of symptoms prior to hospitalization did not alter the found associations, but we cannot discard that others unanticipated confounders were present.

In conclusion, this study presented evidences for a role of age and sex on the risk for progression to severe leptospirosis after infection. Reasons why adults and men have higher risk for progression to severe disease after infection remains to be elucidate, but may involve differences in the *Leptospira* inoculum, presence of antibodies against *Leptospira* acquired during a prior infection and differences in inflammatory and immune response due to immune system maturity or hormone levels. Understanding the mechanisms behind age and sex

influence on the risk for disease progression may have obvious implications for future research on vaccine development and use of anti-inflammatory and immune modulator drugs in the treatment of leptospirosis.

Acknowledgments

We thank team members from the Urban Health Council of Pau da Lima and Oswaldo Cruz Foundation who participated in the data collection for the cohort study; the staff at Hospital Couto Maia for their assistance in conducting the study and most of all the resident associations that comprise the Urban Health Council of Pau da Lima, the community leaders and residents and the patients and their families. This work was supported by the Brazilian National Research Council (grants 300861/1996, 473082/2004, 420067/2005, 305723/2006, 554788/2006, 150176/2007), Oswaldo Cruz Foundation (grant 0250.250.102) and the National Institutes of Health (grants NIAID R01 AI052473, FIC D43 TW00919).The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Table 1. Annual incidence of *Leptospira* infection, severe leptospirosis and mortality from leptospirosis, according to age group.

Age group	Infection			Severe disease			Mortality		
	No. cases[a]	Population	Incidence[b] (95% CI)	No. cases[a]	Population	Incidence[c] (95% CI)	No. cases[a]	Population	Incidence[c] (95% CI)
Total[d]	140	1,300	35.9 (30.3-42.2)	341	2,234,688	5.1 (4.6-5.7)	59	2,234,688	0.9 (0.7-1.1)
5-14 y	18	350	17.2 (10.2-27.0)	11	430,057	0.9 (0.4-1.5)	0	430,057	0.0 (0.0-0.3)
15-24 y	35	328	35.5 (24.9-49.1)	89	557,437	5.3 (4.3-6.5)	9	557,437	0.5 (0.2-1.0)
25-34 y	43	223	64.2 (46.8-85.5)	74	435,680	5.7 (4.4-7.1)	10	435,680	0.8 (0.4-1.4)
35-44 y	23	191	40.2 (25.7-59.7)	71	355,698	6.7 (5.2-8.4)	10	355,698	0.9 (0.4-1.7)
45-54 y	11	140	26.2 (13.2-46.4)	50	224,605	7.4 (5.5-9.8)	13	224,605	1.9 (1.0-3.3)
≥55 y	10	68	49.0 (23.8-88.3)	46	231,211	6.6 (4.9-8.8)	17	231,211	2.5 (1.4-3.9)

[a] Cases identified from 2003 through 2005.

[b] Mean annual incidence is represented as cases per 1,000 population.

[c] Mean annual incidence is represented as cases per 100,000 population.

[d] Total includes all age groups ≥5 years of age.

Table 2. Annual incidence of severe leptospirosis among the population of Salvador and *Leptospira* infection among the Pau da Lima cohort subjects, according to age and sex.

Population	Incidence[a]		Risk Ratio (95%CI)[b]
	Severe disease	Infection	
Total[c]	5.1 (4.6-5.7)	3,589.7 (3,575.7-3,603.9)	1.4 (1.3-1.6)
Males[c]	9.3 (8.3-10.4)	4,531.3 (3,568.4-5,664.1)	2.1 (1.8-2.3)
5-14 y	1.4 (0.6-2.6)	2,766.8 (1,520.7-4,598.7)	0.5 (0.3-1.0)
15-24 y	9.9 (7.8-12.3)	4,328.0 (2,625.5-6,676.4)	2.3 (1.8-2.8)
25-34 y	10.9 (8.4-13.8)	8,560.3 (5,442.5-12,673.6)	1.3 (1.0-1.6)
35-44 y	12.2 (9.3-15.7)	5,025.1 (2,435.7-9,047.0)	2.4 (1.9-3.1)
45-54 y	13.3 (9.6-18.1)	2,816.9 (772.7-7,055.2)	4.7 (3.5-6.4)
≥55 y	13.2 (9.2-18.3)	5,882.4 (1,625.8-1,4381.8)	2.1 (1.8-2.3)
Females[c]	1.4 (1.0-1.8)	2,927.0 (2,275.5-3,702.5)	0.5 (0.4-0.6)
5-14 y	0.3 (0.0-1.1)	736.7 (201.1-1,875.3)	0.4 (0.1-1.7)
15-24 y	1.1 (0.6-2.1)	2,930.4 (1,684.1-4,715.3)	0.4 (0.2-0.7)
25-34 y	1.1 (0.5-2.3)	5,084.7 (3,174.7-7,667.8)	0.2 (0.1-0.4)
35-44 y	1.9 (1.0-3.4)	3,485.3 (1,868.6-5,886.2)	0.5 (0.3-1.0)
45-54 y	2.5 (1.1-4.7)	2,518.0 (1,018.2-5,119.0)	1.0 (0.5-1.9)
≥55 y	2.4 (1.1-4.4)	4,411.8 (1,636.0-9,355.1)	0.5 (0.3-1.0)

[a] Mean annual incidence for severe disease and infection (cases per 100,000 population) were determined based on information obtained during population-based surveillance in Salvador and annual follow up serosurveys of the Pau da Lima cohort, respectively, from 2003 through 2005.

[b] Risk ratios were calculated as the ratio between the rates for acquiring severe disease and infection, multiplied by 1,000.

[c] Includes all age groups ≥5 years of age.

Table 3. Age and sex-adjusted risk of death and pulmonary hemorrhage syndrome among hospitalized cases of severe leptospirosis (N=341).

	Adjusted odds ratio (95% CI)[a]	
Age group	Death[b]	SPHS
Female gender	1.63 (0.78-3.40)	2.46 (1.05-5.75)
Age groups		
5-24 y[c]	1.00	1.00
25-34 y	1.59 (0.61-4.15)	1.22 (0.42-3.55)
35-44 y	1.68 (0.64-4.40)	0.84 (0.26-2.69)
45-54 y	3.49 (1.37-8.92)	1.20 (0.37-3.91)
≥55 y	5.60 (2.24-13.97)	1.87 (0.63-5.60)

NOTE. SPHS, severe pulmonary hemorrhage syndrome.

[a] Odds ratios were adjusted for age and sex.

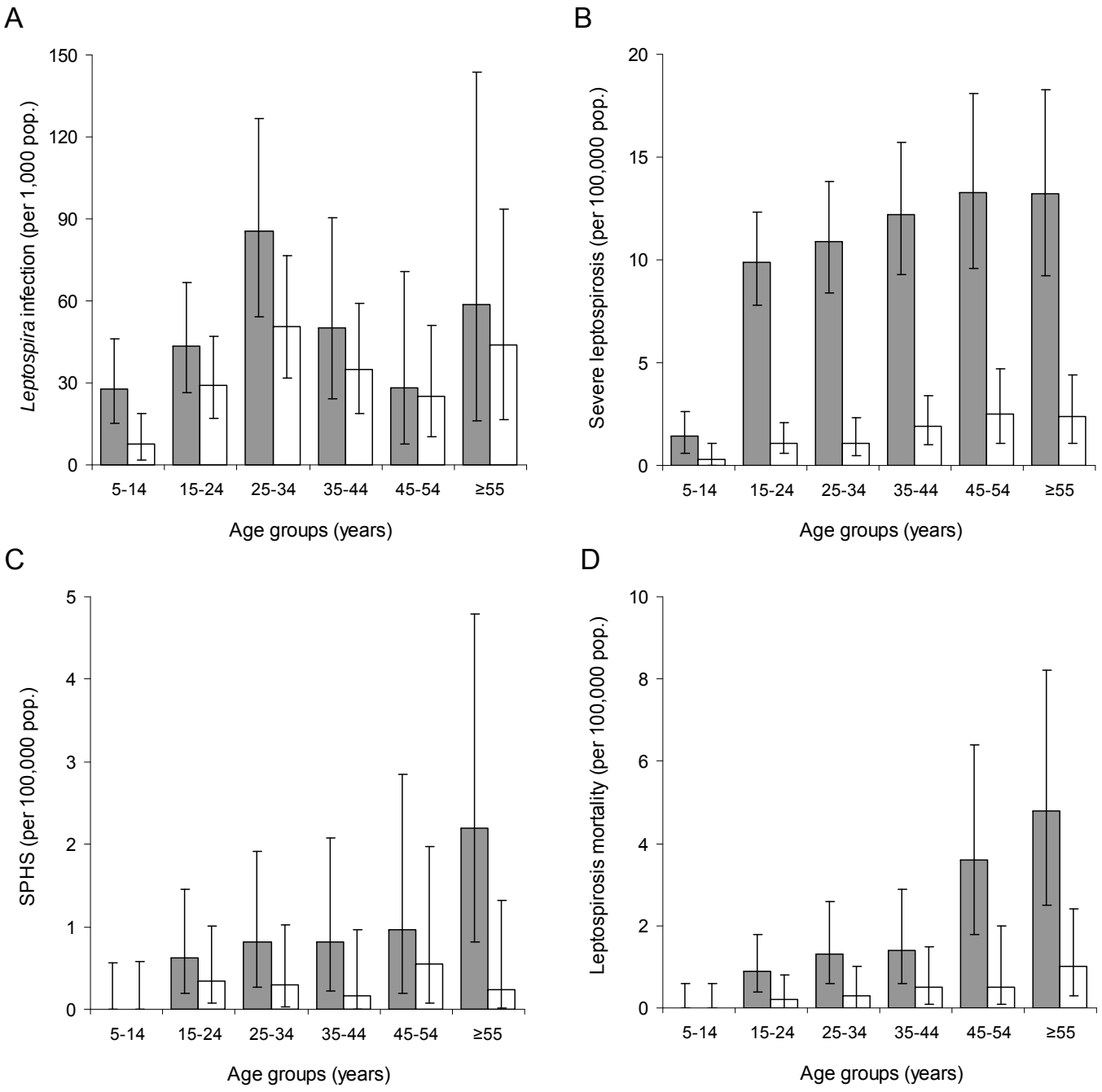
[b] Information on outcome was missing for six patients who were transferred to another hospital.

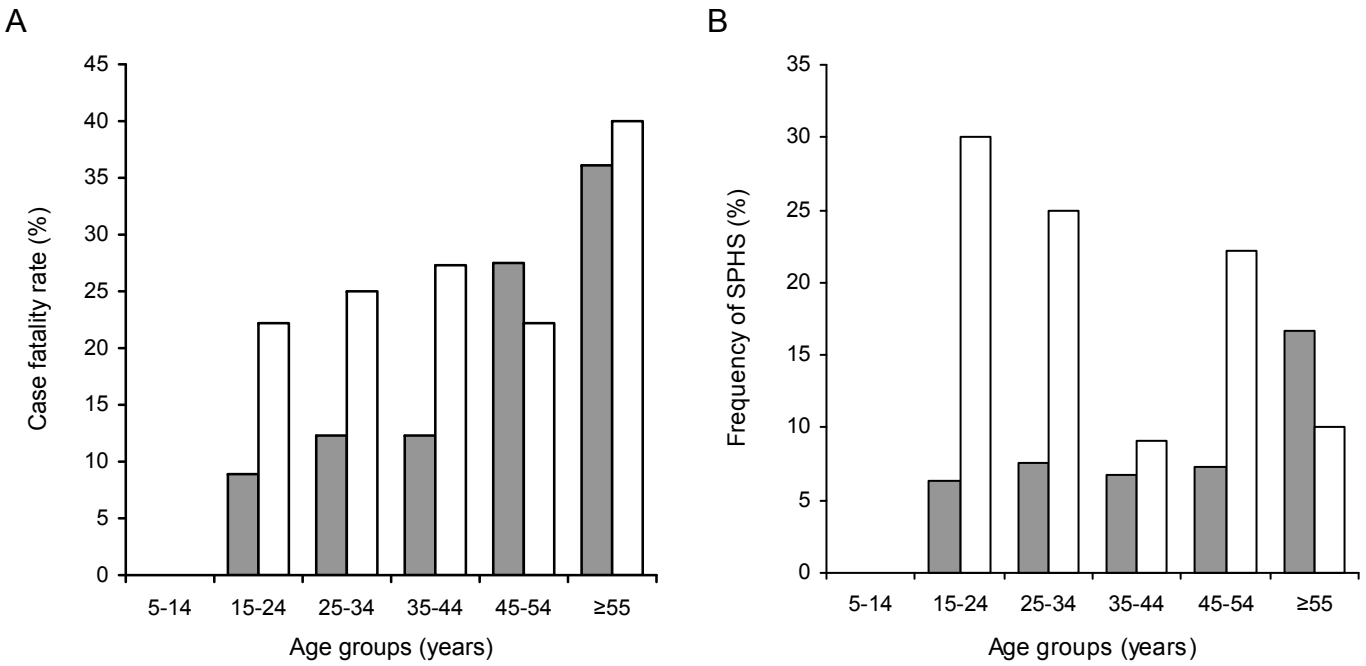
[c] Reference age group was 5-24 years old because there were no cases of death and SPHS in the age group 5-14 years old.

FIGURE LEGENDS

Figure 1. Mean annual incidence of *Leptospira* infection *(A)*, severe leptospirosis *(B)*, leptospirosis-associated severe pulmonary hemorrhage syndrome *(SPHS, C)* and mortality from leptospirosis *(D)*, according to age and sex. Male and female cases are represented as gray and white bars, respectively. Lines represent 95% confidence intervals.

Figure 2. Distribution of the case fatality rate *(A)* and frequency of severe pulmonary hemorrhage syndrome *(SPHS, B)* among cases of severe leptospirosis, according to gender and age group. Male and female cases are represented as gray and white bars, respectively.


A
Leptospira infection (per 1,000 pop.)
150
120
90
60
30
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)
B
Severe leptospirosis (per 100,000 pop.)
20
15
10
5
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)
C
SPHS (per 100,000 pop.)
5
4
3
2
1
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)
D
Leptospirosis mortality (per 100,000 pop.)
10
8
6
4
2
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)


A
Case fatality rate (%)
45
40
35
30
25
20
15
10
5
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)
B
Frequency of SPHS (%)
35
30
25
20
15
10
5
0
5-14
15-24
25-34
35-44
45-54
≥55
Age groups (years)

Supplemental table 1. Characteristics of 341 patients with severe leptospirosis identified during active surveillance in Salvador, Brazil from 2003 through 2005, according to laboratory confirmation status.

Characteristics	**Confirmed or probable cases (n=281)[a]**		**Unconfirmed cases (n=60)**		**p value[b]**
	No. responses	N (%) or mean (SD)	No. responses	N (%) or mean (SD)	
Age (years)	281	34.4 (14.8)	60	41.8 ±15.6	0.001
Male sex	281	241 (86)	60	50 (83)	NS
Days of symptoms	277	6.0 (2.9)	59	5.5 (3.3)	NS
Fever	277	270 (98)	60	57 (95)	NS
Leukocyte count (10^3 cells/mm^3)[c]	280	14.4 (6.2)	57	13.5 (7.4)	NS
Jaundice	280	241 (86)	60	46 (77)	NS
Blood urea nitrogen (mg/dL) [d]	280	153.8 (93.6)	59	134.7 (87.0)	NS
Serum creatinine (mg/dL) [d]	280	4.2 (2.6)	59	3.7 (2.9)	NS
Respiratory insufficiency[e]	281	98 (35)	60	20 (33)	NS
SPHS[f]	281	21 (8)	60	11 (18)	0.009
Death	281	31 (11)	60	28 (47)	<0.001
Paired serum samples	281	232 (83)	60	24 (40)	<0.001

NOTE. NS, not significant. SPHS, severe pulmonary hemorrhage syndrome

[a] A laboratory-confirmed case of leptospirosis was defined as the demonstration of a fourfold microagglutination titer rise between paired serum samples or a microagglutination reciprocal titer ≥800 in one or more serum samples. A laboratory-probable case was defined as having a microagglutination reciprocal titer ≥200 in one or more serum samples.

[b] The chi squared or Fisher exact test, and Student's t-test were used to evaluate for significant differences (P value<0.05) for proportions and continuous data, respectively.

[c] Obtained at the time of hospital admission.

[d] Maximum levels during hospitalization.

[e] Respiratory insufficiency was defined as respiratory rate ≥28 per minute or use of supplemental O_2 therapy.

[f] SPHS was defined as massive pulmonary bleeding and respiratory failure.

6. DISCUSSÃO

Nas últimas décadas, a leptospirose urbana emergiu como um importante problema de saúde pública. Comunidades carentes e sem infra-estrutura de saneamento são as mais atingidas. Embora a cobertura do serviço de esgotamento da cidade de Salvador tenha aumentado de 26% para mais de 80% através de um projeto (Bahia Azul) para reduzir a poluição dos mares da cidade, nem todos os bairros, como Pau da Lima, foram beneficiados pela intervenção. Portanto, estudos para definir fatores de risco para infecção pela *Leptospira* são fundamentais para guiar quais e onde futuras intervenções de prevenção devem ser priorizadas. Além disso, entender porque algumas pessoas desenvolvem leptospirose grave enquanto a maioria apresenta infecções sub-clínicas ou leves pode ter grandes implicações tanto na identificação de pacientes com maior risco de desfechos graves associados à doença, quanto no desenvolvimento de vacinas e drogas capazes de modular a evolução da infecção.

No primeiro manuscrito desta tese, são apresentados resultados de um estudo de corte transversal realizado em uma comunidade carente de Salvador. Nós avaliamos mais de 3.000 residentes da comunidade e identificamos que 15% deles apresentavam evidência sorológica de infecção prévia por leptospiras. O uso de SIG (Sistema de Informação Geográfica) e de abordagens de modelagem permitiram identificar que deficiências de saneamento nesta comunidade, como esgotos abertos, áreas propensas a alagamento e acúmulo de lixo, são fatores de risco para infecção pela *Leptospira*. Além disso, identificamos que baixo nível sócio-econômico está associado a risco de infecção independentemente das condições ambientais. Nossos resultados são limitados pelo desenho de corte transversal e a impossibilidade de identificar temporalidade entre a exposição e o desfecho. Estudos prospectivos estão sendo realizados nesta população e servirão para confirmar nossos achados.

No segundo manuscrito desta tese, nós apresentamos as incidências de infecção pela *Leptospira*, leptospirose grave, SHPL, e mortalidade por leptospirose, de acordo com sexo e idade. As incidências de infecção pela *Leptospira* foram estimadas a partir do seguimento de três anos de uma coorte na comunidade de Pau da Lima em Salvador, e as incidências de leptospirose grave, SHPL, e mortalidade foram estimadas através de vigilância hospitalar para leptospirose em Salvador no mesmo período. Nossos resultados sugerem que a maior carga da leptospirose grave na população de homens adultos se deve inicialmente a um maior risco de infecção neste grupo. Entretanto, aumento na idade e o sexo masculino parecem ter influência ainda maior no risco de progressão para leptospirose grave após a infecção. Além disso, identificamos entre os casos de leptospirose grave que idade ≥45 anos e sexo feminino são fatores de risco para óbito e para SHPL, respectivamente. Estes achados trazem importante contribuição no entendimento da história natural da infecção pela *Leptospira* e reforçam a hipótese de que a resposta imune do hospedeiro é o principal fator responsável pela variedade de manifestações clínicas que ocorrem após a infecção pela *Leptospira*. Futuras investigações serão necessárias para confirmar nossos resultados em outras populações e para determinar os mecanismos patogênicos por trás da influência da idade e do sexo no desenvolvimento de leptospirose grave.

7. CONCLUSÕES

Os resultados apresentados nesta tese sugerem que a prevenção da leptospirose urbana é possível 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. Além disso, a evidência de que indivíduos do sexo masculino e ≥15 anos tem maior risco de desenvolvimento de leptospirose grave após infecção sinaliza para a importância de realização de estudos que esclareçam os mecanismos patogênicos por trás da associação entre idade e sexo com risco de progressão da doença. Este conhecimento pode ajudar a guiar o desenvolvimento de vacinas e de drogas imunomoduladoras capazes de modificar o curso da infecção e prevenir o surgimento de formas graves da leptospirose.

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9. ANEXOS

Manuscritos publicados durante o doutorado relacionado a outras doenças:

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Haemophilus influenzae meningitis 5 years after introduction of the *Haemophilus influenzae* type b conjugate vaccine in Brazil

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Received 15 November 2006; received in revised form 6 March 2007; accepted 13 March 2007
Available online 30 March 2007

Abstract

The long-term impact of *Haemophilus influenzae* type b (Hib) conjugate vaccine, introduced throughout Latin America in the late 1990s, has not been evaluated. Active surveillance for *H. influenzae* meningitis was performed from August 9, 1996 to August 8, 2004 in Metropolitan Salvador, Brazil. Five years after the introduction of Hib conjugate vaccine, Hib meningitis incidence decreased from 2.39 to 0.06 cases per 100,000 population (98%) overall, and from 60.9 to 3.1 cases per 100,000 population (95%) in children <1 year of age. A transient serotype replacement phenomenon was observed associated with a small increase of meningitis due to two *H. influenzae* type a clonal groups. These findings indicate that Hib immunization campaign has led to the virtual elimination of Hib disease in this region.

Keywords: *Haemophilus influenzae*; Vaccine effectiveness; Serotype replacement

1. Introduction

Introduction of *Haemophilus influenzae* type b (Hib) polysaccharide-protein conjugate vaccines have dramatically reduced the burden of Hib invasive disease in the last 20 years [1–3]. Clinical trials demonstrated that conjugate vaccines have an efficacy of 90–100% in preventing Hib invasive disease [4,5]. Furthermore, the vaccine has been highly effective, even in populations where coverage is not complete, due to its ability to reduce nasopharyngeal colonization in immunized individuals [6,7] and induce herd immunity [8,9]. In the United States (US) and most industrialized countries, Hib invasive disease has been virtually eliminated [1–3]. Whereas more than 10,000 cases of Hib invasive disease were reported annually in the US prior to introduction of conjugate vaccines, less than 100 cases are now reported each year [1].

The priority for Hib invasive disease is to extend the benefits afforded by conjugate vaccines to developing countries [2]. Worldwide, in 2003, 61% of children in target age groups for immunization did not receive the conjugate vaccine [10]. Hib diseases remain the cause of an estimated 386,000 deaths yearly [11]. However, major progress has been made in South and Central Americas and the Caribbean where Hib conjugate vaccines were introduced in the late 1990s and are used in all but four countries [12]. Although several studies have

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0264-410X/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2007.03.024

Transactions of the Royal Society of Tropical Medicine and Hygiene (2007) **101**, 1147—1153

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Hospital-based surveillance of meningococcal meningitis in Salvador, Brazil

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Received 21 March 2007; received in revised form 14 June 2007; accepted 14 June 2007
Available online 6 August 2007

KEYWORDS
Meningococcal disease;
Meningitis;
Neisseria meningitidis;
Serogroup;
Epidemiology;
Brazil

Summary This study aimed to describe the clinical, epidemiological and microbiological features of meningococcal meningitis in Salvador, Brazil. Between February 1996 and January 2001, a hospital-based surveillance prospectively identified cases of culture-positive meningococcal meningitis. Demographic and clinical data were collected through interview and medical chart review. Antisera and monoclonal antibodies were used to determine the serogroup and serotype:serosubtype of the isolates, respectively. Surveillance identified a total of 408 cases of meningococcal meningitis, with a case fatality rate of 8% (32/397). The mean annual incidence for the 304 culture-positive cases residing in metropolitan Salvador was 1.71 cases per 100 000 population. Infants <1 year old presented the highest incidence (14.7 cases per 100 000 population). Of the 377 serogrouped isolates, 82%, 16%, 2% and 0.3% were serogroups B, C, W135 and Y, respectively. A single serotype:serosubtype (4,7:P1.19,15) accounted for 64% of all cases. Continued surveillance is necessary to characterise strains and to define future prevention and control strategies.

1. Introduction

Neisseria meningitidis is a common cause of disease worldwide, responsible for significant morbidity and mortality in infants and young children (Bilukha and Rosenstein, 2005). Without appropriate antimicrobial treatment, most cases

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0035-9203/$ — see front matter © 2007 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.trstmh.2007.06.012

Journal of Infection (2008) **57**, 204–213

www.elsevierhealth.com/journals/jinf

Transmission of *Streptococcus pneumoniae* in an urban slum community☆

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Accepted 2 June 2008
Available online 30 July 2008

KEYWORDS
Streptococcus pneumoniae;
Nasopharyngeal carriage;
Pneumococcal conjugate vaccines;
Antibiotic resistance;
Urban slums

Summary *Background:* Inhabitants of slum settlements represent a significant proportion of the population at risk for pneumococcal disease in developing countries.
Methods: We conducted a household survey of pneumococcal carriage among residents of a slum community in the city of Salvador, Brazil.
Results: Among 262 subjects, 95 (36%) were colonized with *Streptococcus pneumoniae*. Children <5 years of age (OR, 8.0; 95% CI, 3.5–18.6) and those who attended schools (OR, 2.7, 95% CI, 1.2–6.0) had significantly higher risk of being colonized. Of 94 isolates obtained from colonized individuals, 51% had serotypes included in the seven-valent pneumococcal conjugate vaccine. Overall, 10% (9 of 94 isolates) were nonsusceptible to penicillin and 28% (27 of 94 isolates) were resistant to cotrimoxazole. BOX-PCR, PFGE and MLST analyses found that 44% of the carriage isolates belonged to 14 distinct clonal groups. Strains of the same clonal group were isolated from multiple members of 9 out of the 39 study households. Nineteen carriage isolates had genotypes that were the same as those identified among 362 strains obtained from active surveillance for meningitis.

☆ This work has not been presented previously in a meeting.

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doi:10.1016/j.jinf.2008.06.017

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