FUNDAÇÃO OSWALDO CRUZ

CENTRO DE PESQUISAS GONÇALO MONIZ

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

TESE DE DOUTORADO

ESTUDOS ECOLÓGICOS SOBRE RESERVATÓRIOS URBANOS DE LEPTOSPIROSE EM SALVADOR

FEDERICO COSTA

Salvador – Bahia – Brasil
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Orientador: Prof. Dr. Mittermayer Galvão dos Reis
Co-orientador: Prof. Dr. Guilherme Sousa Ribeiro

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"ESTUDOS ECOLÓGICOS SOBRE RESERVATÓRIOS URBANOS DE LEPTOSPIROSE EM SALVADOR"

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FOLHA DE APROVAÇÃO

COMISSÃO EXAMINADORA

Dr. Zilton da Araújo Andrade
Pesquisador Titular
CPqGM / FIOCRUZ

Drª Flávia Weykamp da Cruz McBride
Professor Adjunto
UFBA

Dr. José Carlos Miranda
Pesquisador Associado
CPqGM / FIOCRUZ

A São Salvador da Bahia de Todos os Santos

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RESUMO

Introdução: A leptospirose é um importante problema de saúde urbana devido às epidemias anuais que ocorrem em comunidades carentes e à alta mortalidade associada às formas graves. Os ratos são considerados os principais reservatórios na transmissão urbana. Entretanto, não existem estudos que sistematicamente definam os fatores de infestação por ratos e as características ambientais que influenciam o risco de transmissão da leptospirose. **Objetivos**: 1) Determinar a associação entre infestação por roedores e infecção por *Leptospira* em um estudo de coorte prospectivo realizado em uma comunidade carente de Salvador-BA. 2) Desenvolver e validar um escore domiciliar baseado em características da infestação de ratos para predizer o risco de leptospirose em Salvador. **Métodos**: Para o objetivo 1 realizou-se um estudo de caso-controle aninhado numa coorte longitudinal onde foram definidos como domicílios-casos aqueles que tiveram um ou mais indivíduos com infecção assintomática por *Leptospira.* Controles foram domicílios aleatoriamente selecionados daqueles que tiveram indivíduos sem infecção. Avaliaram-se domicílios registrando sinais de infestação por roedores e características ambientais e foi realizada regressão logística para identificar fatores de risco para infecção. Para o objetivo 2 desenvolveu-se um estudo caso-controle 1:2, onde domicílios-casos foram aqueles nos quais residiam pessoas (casos) com leptospirose. Utilizou-se metodologia similar a do objetivo 1 para avaliar e analisar fatores de risco. Adicionalmente foi desenvolvido um escore preditivo baseado no modelo de regressão logística que foi validado num grupo independente de domicílios-casos e controle. Utilizaram-se curvas características de recepção (ROC) para analisar o desempenho preditivo do escore. **Resultados**: Objetivo 1: Registrou-se elevado nível de infestação por *Rattus norvegicus* (>45%). Identificaram-se como fatores de risco de infecção: fezes de *R. norvegicus* (OR 4.6 IC 95% 1.9-10.7), tocas (OR 2.8, IC 95% 1.1-7.3), parede do domicilio sem reboco (OR 2.5, IC 95% 1.1-7.4) e renda domiciliar per capita (OR 0.9 por US$/dia, IC 95% 0.8-0.9). Objetivo 2: Achamos uma elevada proporção (>44%) de domicílios infestados. Os fatores de risco independentes para leptospirose foram tocas, fezes de *Rattus norvegicus*, trilhas, casa abandonada <10m e domicílio sem reboco. Designaram-se valores de escore para cada fator de risco (3, 3, 2, 2 e 2 respectivamente). A área sob a curva ROC foi 0,70 (IC95%, 0,64-0,76) para o grupo de desenvolvimento e 0,71 (95; 0,65-0,79) para o de validação. **Conclusões**: Foi identificada uma elevada proporção de domicílios infestados com *R. norvegicus*. Os fatores de risco para infecção por *Leptospira* e leptospirose grave foram similares. Foi definido e validado um escore preditivo que identifica domicílios de elevado risco dentro de comunidades com transmissão endêmica de leptospirose. Estes achados sugerem que a triagem da infestação por roedores e a identificação de domicílios de risco, podem constituir ações de uma estratégia recomendável para dirigir intervenções de controle de roedores em populações de risco.

Palavras-chave: Leptospirose, Transmissão de doença infecciosa, Áreas de pobreza,

Roedores, Fatores de risco, Escore preditivo.

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ABSTRACT

Background: Leptospirosis is a relevant problem of urban health because of the epidemics occurring in cities throughout the developing world and the high mortality associated with severe disease. In urban areas, leptospirosis is transmitted to humans by the rodent *Rattus norvegicus*. However, there are no studies that systematically defined rodent infestation factors and environmental characteristics that influence the risk for Leptospirosis transmission. **Aims:** 1) To identify environmental risk factors for asymptomatic or subclinical *Leptospira* transmission. 2) To develop and validate a household score based on rodent infestation characteristics to predict leptospirosis risk in Salvador. **Methods:** For aim 1 a nested case-control study was conducted in the study site. A household was regarded as a case household if at least one new *Leptospira* infection occurred among cohort subjects. Control households were randomly selected and households were surveyed for signs of rodent infestation and environmental characteristics. We used conditional logistic regression modeling to identify risk factors for *Leptospira* infection. For aim 2 we developed a case-control study (1:2), where case households were households in which leptospirosis cases resided. Control households were located within 30m of a case-household. We used similar methodology to that in aim 1 to identify and analyze risk factors for leptospirosis. Additionally, we used the logistic regression model to develop a predictive score for leptospirosis that was validated in an independent group of cases and control households. We used receiver operating characteristic (ROC) curve analysis to evaluate the performance of the prediction score. **Results:** Aim 1: we identified a high level of *R. norvegicus* infestation (>45%). We identified the following independent risk factors: *R. norvegicus* feces (OR 4.6 CI 95% 1.9-10.7), burrows (OR 2.8, CI 95% 1.1-7.3), unplastered walls (OR 2.5, CI 95% 1.1-7.4) and household per capita income (OR 0.9 for each US$ per day increase, CI 95% 0.8-0.9). Aim 2: more than 44% of the households presented rodent signs. Independent risk factors for acquiring leptospirosis in a household were rodent burrows, *Rattus norvegicus* feces, rodent runs, household bordering an abandoned house, and unplastered walls. A prediction score was developed by assigning points (3, 3, 2, 2 and 2 respectively) to each risk factor. The area under the ROC curve for the scoring system was 0.70 (95% CI, 0.64-0.76) and 0.71 (0.65-0.79) for the development and validation datasets. **Conclusions:** Our study indicates that high proportions of urban slum households are infested with *R. norvegicus*. Risk factors for asymptomatic *Leptospira* infection and severe leptospirosis were similar. The prediction score showed good performance in identifying high-risk households for leptospirosis. These findings suggest that community-based screening for rodent infestation can be a strategy to target rodent and environmental control measures to populations at highest risk for leptospirosis

Keywords: Leptospirosis, transmission, urban slums, rodents, risk factors, predictive score.

LISTA DE ABREVIATURAS

ArcGIS	Programa para análises de dados espaciais
CCZ	Centro de Controle de Zoonoses
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
GAM	Modelos aditivos generalizados
GIS	Sistema de Informação Geográfica
IQR	Intervalo interquartil
MAT	Teste de microaglutinação
NS	Não significante
PCR	Reação em cadeia da polimerase
R	Programa para análises de dados estatísticos
SAS	Programa para análises de dados estatísticos
SD	Desvio padrão
SIG	Sistema de Informação Geográfica
WHO	Organização Mundial de Saúde

SUMÁRIO

1. INTRODUÇÃO

1.1. *Leptospira* e leptospirosis

Aspecto histórico

A leptospirose é uma zoonose que afeta uma ampla variedade de animais domésticos e silvestres assim como também ao homem (FAINE et al., 1999). Alguns destes animais atuam como reservatórios crônicos do agente etiológico *Leptospira*. A doença foi primeiramente conhecida como uma "infecção ictérica", que afetava geralmente tropas militares, trabalhadores nas redes de esgoto, mineiros e agricultores de arroz. Existe consenso de que a doença foi identificada como uma entidade desde a antiguidade (FAINE SB, 1999). Entretanto acredita-se que a síndrome clínica foi descrito adequadamente pela primeira vez por Larrey em 1812 (LARREY, 1812). Nos seguintes cinquenta anos descrições adicionais foram registradas principalmente relacionadas às epidemias (GRIESINGER, 1853; LANDOUZI, 1883a; b; MATHIEU, 1886). Em 1886, A. Weil descreveu a doença e chamou a atenção sobre o comprometimento hepático e renal. Assim, os casos humanos de leptospirose com icterícia e comprometimento hepatorenal são denominados atualmente como síndrome de Weil (WEIL, 1886). Uma vez descrita a doença e durante a primeira década do século XX, casos de leptospirose humana foram reportados frequentemente na Europa e outros países (CAVATORTI, 1903; CHOWDRY, 1903; DE LUNA, 1903; EINHORN, 1904; KLODNITSKI, 1906; LEBREDO ;MARTINEZ, 1905; SANDWITH, 1904a; b; STIMSON, 1907; WILKINSON, 1906).

Em 1915, Ido, Inada e colaboradores (IDO ;HOKI, 1915; IDO ;HOKI ;ITO, 1915; INADA ;HOKI ;IDO, 1915) no Japão e Frome e Hübener (HÜBENER ;REITER, 1915; UHLENHUTH ;FROME, 1915) na Alemanha reportaram o isolamento de um agente etiológico através da infecção de cobaios com tecidos de pacientes graves e constataram que a sintomatologia da doença podia ser reproduzida neste roedor. Este organismo foi descrito e nomeado por pesquisadores japoneses como *Spirochaeta icterohaemorrhagiae* (INADA ;IDO, 1915). O primeiro estudo completo sobre patogenicidade, diagnose, sintomatologia, profilaxia e tratamento foi publicado por Inada et al. (INADA et al., 1915; 1916). Simultaneamente, pesquisadores alemães isolaram também o organismo e denominaram-no *Spirochaeta nodosa* (UHLENHUTH ;FROME, 1915).

Nessa mesma época Miyajima (MIYAJIMA, 1916) relatou, durante o curso das suas investigações sobre a doença tsugamushi (uma forma de tifo), ter achado espiroquetas semelhantes a *Spirochaeta icterohaemorrhagiae* nos rins de um roedor rural, *Microtus montebelloi*. Emseguida, em 1917, Ido et al. (IDO et al., 1917) discutem a importância do papel dos roedores como reservatórios da doença. Achados similares foram reportados mais tarde por Noguchi (NOGUCHI, 1917) nos Estados Unidos. Nesta época, Noguchi realizou a primeira publicação sistemática descrevendo as características morfológicas, como a forma helicoidal apertada do corpo, propôs um novo gênero: *Leptospira*.

Taxonomia

A taxonomia e a classificação das leptospiras são complexas e vêm experimentando modificações nos últimos anos. Sucintamente, as leptospiras pertencem à Ordem Spirochaetales, a qual faz parte de um filo bacteriano único, Spirochaetes. A família Leptospiraceae compreende o gênero *Leptospira*, o qual é composto por bactérias saprófitas e patogênicas. Atualmente, as leptospiras patogênicas são classificadas em sete espécies (FAINE et al., 1999; FARR, 1995; LEVETT, 2001) antigenicamente diversas, sendo subdivididas em mais de 200 sorovares (BHARTI et al., 2003; LEVETT, 2001; NALAM et al., 2010).

Microbiologia

O nome leptospira tem sua origem em duas palavras gregas *leptós*, fino, pequeno, delicado e *speira*, espira. As leptospiras são bactérias helicoidais de 6 a 20 µm de comprimento e 0,1 µm de diâmetro, com as extremidades em forma de ganchos. As leptospiras são espiroquetas móveis, obrigatoriamente aeróbicas com características de bactérias Gram-negativas. Crescem em condições ótimas de temperatura de 28 a 30ºC e pH 7,2 a 7,6, são de difícil cultivo necessitando de meios especiais. As leptospiras são bastante sensíveis ao ressecamento, desinfetantes, extremos de temperatura e pH inferior a 6,8 ou superior a 8,0 (FAINE et al., 1999). No entanto, sobrevivem na água e em cultura por longos períodos (TRUEBA et al., 2004), bem como em solos, lama, acúmulos de água doce e rios (HENRY ;JOHNSON, 1978).

Ciclo

O ciclo da leptospirose na natureza mostra que a transmissão é mantida por diversas espécies de mamíferos que servem de reservatórios para a *Leptospir*a. Quando infectadas, as

espécies dos reservatórios apresentam colonização persistente dos túbulos proximais renais e disseminam de forma assintomática o organismo para o ambiente através da urina (BHARTI et al., 2003; LEVETT, 2001). Entre reservatórios da mesma espécie a transmissão ocorre por contato direto entre os animais e é comumente observado um aumento da prevalência da infecção com a idade (LEVETT, 2001). A infecção nos humanos é considerada acidental e acontece por contato direto ou indireto da pele não íntegra ou de superfícies mucosas com a urina de um animal infectado (LEVETT, 2001).

Manifestações clínicas

Apos a infecção de um hospedeiro humano por leptospiras patogênicas o período de incubação médio é de 7 a 14 dias. Existe uma grande variedade de manifestações clínicas que vão desde uma infecção subclínica seguida de soroconversão, até duas formas clínicas bem reconhecidas. A primeira é de uma doença febril aguda autolimitada, e a segunda é de uma doença grave e potencialmente letal que pode se apresentar como insuficiência renal aguda, icterícia, sangramentos e pneumonite em diferentes combinações (BHARTI et al., 2003; LEVETT, 2001; 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 relacionada a pneumonite e sangramento pulmonar é denominada “síndrome de hemorragia pulmonar” e apresenta letalidade >50% (GOUVEIA et al., 2008). Ainda que não esteja claramente estabelecido, estima-se que 90-95% das pessoas infectadas, que apresentam manifestações clínicas da leptospirose, desenvolvem a forma leve e autolimitada da doença, entretanto 5-10% evoluem para as formas graves (BHARTI et al., 2003; LEVETT, 2001).

Aspectos históricos das epidemias

As causas da maioria dos surtos históricos podem nunca vir a ser provadas. Entretanto, a limitada evidência disponível não tem dissuadido os historiadores de especular sobre as causas das aflições desde tempos remotos (Marr, 2010). Surtos de febre e icterícia potencialmente ocasionados por leptospirose têm sido relacionados à estação e a áreas geográficas (VAN THIEL, 1948). Na década seguinte, na Europa, no Japão e na Austrália, estas febres foram associadas às ocupações das pessoas e a ambientes específicos (surtos de doença de cortadores de cana, doença do rebanho de porco, febre da lama) muito antes da etiologia ser descrita (TORTEN, 1979b; VAN THIEL, 1948). Surtos históricos têm sido descritos durante conflitos bélicos como a guerra civil americana (NEILL, 1918) e a primeira

guerra mundial. Levett (LEVETT, 2001) realizou um levantamento de estudos de surtos relacionados a águas contaminadas a maioria dos quais estava relacionada a atividades recreativas como a natação e/ou banhos em rios e lagos. As publicações a respeito de epidemias sugerem que as formas de infecção subclínica são muito frequentes e que a doença pode apresentar dimensões maiores do que é conhecido.

Mudança do padrão rural para padrão urbano

Enquanto surtos recentes têm ocorrido no ambiente rural (BHARTI et al., 2003; LEVETT, 2001), um novo padrão epidemiológico tem emergido no ambiente urbano (KARANDE et al., 2002; KO et al., 1999; LAROCQUE et al., 2005; ROMERO ;BERNARDO ;YASUDA, 2003; SARKAR et al., 2002; TASSINARI et al., 2004). A mudança nos padrões epidemiológicos da leptospirose é atribuída a alterações no processo de ocupação ambiental. O Brasil é um clássico exemplo desta mudança, visto que a intensa migração da população humana do meio rural ao meio urbano nas décadas de 1950, 1960 e 1970 proporcionou um crescimento desordenado das cidades. A instalação de imigrantes pobres nas cidades levou ao aparecimento de numerosas e populosas áreas de pobreza conhecidas como favelas, com problemas de saneamento básico, inexistência de escoamento de esgotos adequado, falta de coleta de lixo e aumento da população de ratos. As condições climáticas, como altas temperaturas e precipitações, somadas às precárias condições de saneamento básico e à falta de conhecimento da população sobre medidas de higiene e de prevenção básica da saúde modificaram as características da leptospirose, que passa de uma doença esporádica e ocupacional, para uma doença com características ecológicas próprias das capitais mais populosas, atingindo as populações de baixa renda durante a época de chuvas.

Epidemias urbanas no Brasil

No Brasil, a leptospirose tem causado epidemias urbanas anualmente, e durante períodos sazonais de chuvas fortes. Estas epidemias primariamente afetam os mesmos grupos de risco dentro das comunidades mais pobres (favelas) (KO et al., 1999; ROMERO ;BERNARDO ;YASUDA, 2003; TASSINARI et al., 2004). Apenas no Brasil, 10.000 casos de leptospirose grave são notificados anualmente durante epidemias que ocorrem em todas as principais cidades do país (SECRETARIA DE VIGILÂNCIA SANITÁRIA / MINISTÉRIO DA SAÚDE DO BRASIL, 2007). Estudos no Brasil identificaram a *L. interrogans* sorovar copenhageni como o agente etiológico destas epidemias urbanas (BAROCCHI et al., 2001;

KO et al., 1999). Este sorovar está comumente associado a reservatórios do gênero *Rattus* (BHARTI et al., 2003; VINETZ et al., 1996).

1.2. Reservatórios da leptospirose

Distribuição e diversidade

A leptospirose é considerada a zoonose mais difundida no mundo, devido à habilidade do patógeno de induzir um estado de portador em uma gama extensa de animais (FAINE et al., 1999). A bactéria tem sido isolada em animais de varias ordens, como Insectivora, Chiroptera, Rodentia, Lagomorpha, Carnívora e em animais ungulados (HARTSKEERL ;TERPSTRA, 1996). Cada sorovar tende a ser mantido por um hospedeiro animal específico, sendo então este sorovar considerado adaptado a determinada espécie animal. 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 (BHARTI et al., 2003; LEVETT, 2001). Listas de sorovares e os animais aos quais eles foram associados têm sido publicadas desde 1966 (ALEXANDER et al., 1966; TORTEN, 1979a).

Reservatórios silvestres e domésticos

Os tipos de reservatórios animais são os silvestres, os domésticos e os sinantrópicos. Os principais reservatórios silvestres são os pequenos mamíferos, os répteis e os anfíbios. O papel que as aves e os insetos desempenham como reservatórios da leptospirose ainda não foi esclarecido, no entanto, é possível que contribuam para a transmissão da leptospirose (FAINE et al., 1999).

Nos animais domésticos a leptospirose pode assumir duas fases distintas, a fase aguda (dividida em uma fase septicêmica e uma fase imunológica) e a fase crônica (leptospiúrica). Na fase imunológica existem animais que podem apresentar sinais clínicos da doença, como febre, hemorragia, hemoglobinúria, icterícia, abortos e sinais nervosos, podendo chegar à morte por insuficiência hepática ou renal, enquanto em outros animais a doença não ocasiona sinais clínicos, passando muitas vezes despercebida. Após a fase septicêmica, as leptospiras alojam-se em vários sítios teciduais, dentre eles os túbulos renais, e os animais começam a eliminar leptospiras, através da urina, constituindo a fase leptospiúrica. Nos reservatórios silvestres e sinantrópicos não existem registros dos animais que apresentam sinais clínicos da doença, no entanto, eles eliminam leptospiras no ambiente, por semanas, meses ou por toda a vida.

Roedores sinantrópicos

Dentre os reservatórios sinantrópicos os roedores são os reservatórios habituais das leptospiras no ambiente urbano. Acredita-se que entre os roedores sinantrópicos o rato de esgoto (*Rattus norvegicus*) tem o papel de maior risco com relação à transmissão da leptospirose no ambiente urbano já que numerosos estudos têm identificado esta espécie como o reservatório predominante de *Leptospira* na cercania dos domicílios de pessoas com a doença (DE FARIA et al., 2008; MATTHIAS et al., 2008; PEZZELLA et al., 2004; VINETZ et al., 1996). Adicionalmente, os casos graves de leptospirose nas maiores cidades Brasileiras são causados predominantemente por um único sorovar *L. interrogans* sorovar *Copenhageni*, o qual está associado a *R. norvegicus* (BAROCCHI et al., 2001; KO et al., 1999; PEREIRA et al., 2000; ROMERO ;YASUDA, 2006).

Roedores e peridomicílio

Resultados de estudos realizados em Salvador (Bahia) sugerem que o ambiente peridomiciliar contaminado pela urina dos ratos infectados, seja um local com grande probabilidade para a transmissão da leptospirose durante as epidemias urbanas. Um estudo caso-controle identificou que marcadores subjetivos da infestação de roedores, como por exemplo, enxergar ratos na proximidade do domicílio e residência próxima de ambientes que favorecem a presença de roedores, foram ambos fatores de risco independentes para leptospirose grave (SARKAR et al., 2002). Adicionalmente um estudo de corte transversal identificou os mesmos fatores de risco associados à presença de anticorpos contra *Leptospira* (REIS et al., 2008).

1.3. Justificativa: barreiras na prevenção da leptospirose

Nas áreas urbanas, as populações com baixas condições sócioeconômicas estão mais vulneráveis à doença. As medidas utilizadas não têm se mostrado efetivas e isto pode ser comprovado pelo alto índice de morbimortalidade da leptospirose, comprovando a necessidade de desenvolver medidas preventivas para otimizar recursos humanos e materiais, mantendo uma boa relação custo-benefício. Diversos aspectos da doença limitam a prevenção da leptospirose, entre as quais os mais relevantes são:

Técnicas de diagnóstico laboratorial

O diagnóstico da doença deve ser feito baseado em elementos de ordem epidemiológica associado a manifestações clínicas sugestivas, e confirmação laboratorial. O

diagnóstico laboratorial pode ser feito por visualização direta, por isolamento do organismo, por testes sorológicos ou por métodos moleculares (LEVETT, 2001). A visualização direta das leptospiras em sangue ou urina é feita através da microscopia de campo escuro, entretanto, este método possui baixa sensibilidade e especificidade e é pouco empregado. A visualização direta do microorganismo em tecidos é tradicionalmente realizada pela coloração com prata. Amostras de sangue, urina e líquor são utilizadas para cultura e isolamento do organismo. Na prática, a maioria dos casos de leptospirose é diagnosticada por sorologia. A microaglutinação (MAT) é considerada o teste padrão (FAINE et al., 1999) e baseia-se na identificação por microscopia de campo escuro de aglutinação do soro do paciente com antígenos vivos de diferentes sorogrupos da *Leptospira*. A MAT é uma técnica de difícil execução e é realizada apenas em laboratórios especializados. Para a MAT, é necessário o uso de bactérias e amostras pareadas para a confirmação do aumento do título de anticorpos e diagnóstico da doença (LEVETT, 2001). Outros testes sorológicos comumente utilizados incluem hemaglutinação indireta e ELISA (Enzyme Linked Immuno Sorbent Assay). Técnicas de biologia molecular como PCR (reação em cadeia da polimerase) 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 desenvolvimento de uma nova tecnologia diagnóstica para a leptospirose, como o teste rápido, que seja simples e capaz de identificar as fases iniciais da doença é uma prioridade na pesquisa em leptospiras (CRODA et al., 2007).

Antibióticos e terapias de suporte

As práticas atuais de tratamento não têm obtido êxito na redução da letalidade das formas graves da doença Os pacientes com leptospirose recebem tratamento baseado no uso de antibióticos e de terapias de suporte (BHARTI et al., 2003; LEVETT, 2001; MCBRIDE et al., 2005). Permanece controverso se o uso de penicilina na hospitalização traz benefício com relação à morbidade/letalidade da leptospirose severa (GUIDUGLI ;CASTRO, 2000), mas a utilização de antibióticos foi relacionada à redução da duração da febre, ao tempo para normalização da função renal e ao tempo de hospitalização (MCCLAIN ;BALLOU, 1984 ; WATT ;PADRE, 1988). Hidratação vigorosa, monitorização em unidade de terapia intensiva (UTI), utilização de medicamentos vasoativos, ventilação mecânica e diálise são indicados como medidas de suporte (MCBRIDE et al., 2005). Apesar das medidas agressivas de suporte e terapia com penicilina acima descritas, a letalidade permanece >10%. A falta de opções de

tratamento efetivas requer que intervenções para leptospirose severa focalizem-se em prevenção.

Vacina para humanos

As vacinas disponíveis para humanos são baseadas em extrato bruto da bactéria e só protegem contra os sorotipos contidos na mesma. Estas vacinas possuem alguns graves inconvenientes, como aparecimento de efeitos colaterais, proteção de curta duração e proteção incompleta contra outros sorovares não presentes na formulação. A utilização em humanos é realizada em alguns países como Cuba (MARTINEZ R, 2004) e China (YAN Y, 2003), sendo restrita a pessoas em atividades ocupacionais de risco. A identificação de vacinas que confiram imunidade protetora de longa duração e imunidade cruzada contra diversos sorovares de *Leptospira* é uma das prioridades no campo da prevenção da leptospirose (BHARTI et al., 2003; BRANGER et al., 2005; SILVA et al., 2007).

Quimioprofilaxia

Para indivíduos com elevado risco de contaminação, é recomendada a administração de antibióticos profiláticos (pré-exposição) (GONSALEZ et al., 1998; SEHGAL et al., 2000 ; TAKAFUJI et al., 1984). As populações mais indicadas a receber esse tipo de tratamento são militares, bombeiros e profissionais que irão se submeter à situação de risco por um tempo limitado. A doxiciclina é o antibiótico mais recomendado devido a sua comprovada eficácia na proteção de indivíduos expostos. A profilaxia secundária (pós-exposição) ainda é controversa, mas tem sido utilizada como medida individual em acidentes de laboratório e em nível populacional após exposições de risco extremo como alagamentos. Alguns autores recomendam administrar doxiciclina para estes indivíduos que já foram expostos às situações de risco extremo (FAINE et al., 1999).

Equipamentos de proteção individual

A utilização de vestimentas e elementos de proteção como luvas e botas é adotada em grupos com atividades ocupacionais de risco. Entretanto, intervenções que previam o uso destes equipamentos em populações de risco em áreas urbanas resultaram difíceis de serem efetivadas pelo seu elevado custo. Outra limitação é a falta de aceitação por parte de alguns grupos como trabalhadores rurais relacionados à colheita de arroz, especialmente em países tropicais (FAINE et al., 1999).

Controle de roedores reservatórios

Neste cenário, a maior parte das intervenções de prevenção focalizam-se no controle químico de reservatórios. As ações respondem a três tipos de situações, bloqueio de casos de leptospirose, tratamento de pontos de risco e resposta a reclamos (CCZ-SP, 2003). O bloqueio dos casos de leptospirose inclui uma única intervenção de tratamento químico nas imediações e no peridomicilio da residência de pacientes confirmados da doença. As atividades relacionadas aos pontos de risco são de nível focal e descontínuas, antes do período de chuva, incluindo tratamento químico em grandes lixões, córregos e em outros lugares considerados de risco para a transmissão da doença, visto que favorecem a presença de reservatórios (CCZ-SP, 2003). O atendimento a reclamos é realizado após recepção de ligação telefônica notificando infestação de roedores por moradores e instituições independentemente da localização geográfica ou risco epidemiológico. Em conjunto, estas intervenções descontínuas e baseadas na aplicação de rodenticida não são eficazes em longo prazo no controle das populações de roedores (CHANNON ;COLE ;COLE, 2000). Adicionalmente, possuem contraindicações, como a geração de resistência aos químicos pelos roedores e o impacto em espécies não alvo (HATHAWAY ;BLACKMORE ;MARSHALL, 1981). O efeito é potenciado pela falta de informação com relação ao controle de roedores, o que favorece a utilização de produtos ilegais como o popular “chumbinho” (dados não publicados), um inseticida anticolinesterásico de alta letalidade (CALDAS et al., 2008).

1.4. Manejo integrado de roedores e controle da leptospirose

Manejo integrado de roedores no mundo e no Brasil

Atualmente é postulado que o controle de roedores deve ser feito dentro das regras do Manejo Integrado de Pragas (MIP) (BENNETT ;OWENS ;CORRIGAN, 2005). Do ponto de vista do MIP, as pragas (reservatórios de doenças) são controladas através do manejo do ambiente. Para que um programa de MIP tenha êxito, o comportamento e a ecologia das espécies alvo, o ambiente em que a praga está ativa, e as mudanças periódicas que ocorrem no ambiente (incluindo as pessoas que compartilham esse ambiente) devem ser levadas em consideração (DAVIS ;CALVET ;LEIRS, 2005; HOLT ;DAVIS ;LEIRS, 2006). Além disto, a segurança das pessoas, do ambiente, e dos animais não alvo, tais como animais de estimação, pássaros, animais domésticos e silvestres, devem também ser considerados (CDC, 2006). No nível operacional o MIP propõe intervenções em três etapas: uma avaliação inicial, a intervenção propriamente dita e uma avaliação final de uma amostra da população com a

finalidade de medir o impacto da intervenção. Estas estratégias estão baseadas na inspeção ambiental do peridomicílio na procura de informações sobre a infestação de roedores e de deficiências ambientais que suportam estas populações. Desde o ano de 2005, algumas das principais cidades do Brasil como São Paulo, Salvador, Recife e Curitiba, têm implementado ações sistemáticas de controle de roedores baseadas neste sistema (BRASIL, 2007; DE MASI ;VILACA ;RAZZOLINI, 2009). As campanhas para a redução da infestação de roedores incluem a aplicação de rodenticidas e em menor escala intervenções ambientais para reduzir fontes de alimento, água e refúgio para os roedores (BRAZIL, 2002). Esforços e metodologias variam de cidade para cidade, mas a maioria dos programas priorizam áreas definidas como de elevada incidência da doença (BRASIL, 2007). Estas áreas são grandes (20.000 a 60.000 domicílios) e apresentam uma forte heterogeneidade socioeconômica e ambiental entre domicílios. Estes programas consomem grandes quantidades de energia e recursos já que requerem inspeção ambiental e tratamento de todos os domicílios dentro das áreas selecionadas três vezes ao ano.

Metodologia para o controle de roedores em áreas de pobreza

Atualmente, o monitoramento do nível de infestação e condições ambientais está baseado em metodologias desenvolvidas nos Estados Unidos e Europa (CDC, 2006; DEFRA, 2005). Estas ferramentas não foram validadas em países em desenvolvimento e com outras características climáticas e geográficas. Especialmente em áreas onde a leptospirose é endêmica, com características de elevada densidade humana, padrão de construção desordenado, e com deficiências de infraestrutura e saneamento básico, o que facilita a existência de fontes adicionais de alimento e refúgio para roedores. Há ainda outro obstáculo para desenvolver medidas preventivas, que é a falta de conhecimento em nosso meio sobre os reservatórios urbanos de *Leptospira*, a sua ecologia e o seu papel na dinâmica de infecção e doença (DE FARIA et al., 2008). Estes conhecimentos são fundamentais para dar suporte às estratégias de controle de reservatórios

Eficácia do manejo de roedores no controle da leptospirose

A partir do ano de 2005, o principal objetivo das cidades que têm implementado programas de controle de roedores é a redução da incidência da leptospirose (COVISA, 2005). Estes programas têm-se mostrado efetivos na diminuição do nível da infestação de roedores dentro das áreas tratadas (BRASIL, 2007; DE MASI ;VILACA ;RAZZOLINI, 2009), porém não existe evidência de que tenham impacto na incidência da leptospirose. O

tamanho das áreas tratadas exige planejamento, implementação e avaliação complexos. Uma limitação para a avaliação do programa é a falta de unidades geográficas compatíveis entre os programas de controle de roedores e o sistema de notificação de casos de leptospirose (REIS et al., 2009). Adicionalmente, devido ao sistema de notificação passiva da doença é registrada uma forte subnotificação o que dificulta a estimação da incidência real da doença e a sua relação com as intervenções de controle de roedores.

Estratificação de risco para leptospirose

Uma das limitações nos programas urbanos de controle de roedores é a falta de indicadores de infestação próprios para áreas de risco de leptospirose. A utilização destas ferramentas não tem sido realizada em um contexto epidemiológico que permita avaliar o seu valor preditivo para risco de infecção e/ou doença. Adicionalmente, não existem estudos que sistematicamente avaliem se variáveis coletadas durante as inspeções da infestação por roedores podem ser utilizadas como marcadores preditivos do risco de transmissão para letospirose. Intervenções estrategicamente dirigidas e com maior custo-benefício levadas a cabo em domicílios com maior risco de infecção por *Leptospira* podem melhorar tanto a eficácia no controle de roedores como a prevenção da doença.

2. OBJETIVOS

2.1 Geral

Identificar e validar marcadores de infestação de roedores que predizem o risco de infecção por *Leptospira* e leptospirose**.**

2.2 Específicos

- Determinar a associação entre infestação de roedores e a infecção humana por *Leptospira*, identificada em uma coorte prospectiva.

- Desenvolver e validar um escore domiciliar baseado em características da infestação de roedores para predizer o risco de leptospirose grave em um estudo caso-controle.

3. MANUSCRITO 1

COSTA, F.; REIS, R.; SANTOS, N.; RIBEIRO, G. S.; FELZEMBURGH, R. D. M.; FRAGA, D. B. M.; ARAUJO, W.; SANTANA, C.; REIS, M. G.; KO, A. I. Rat infestation and insidence of leptospirosis infection in urban slums of Brazil. (Artigo a ser submetido)

RESUMO:

Introdução: A leptospirose é um grave problema de saúde urbana devido às epidemias anuais que ocorrem em comunidades carentes e à alta mortalidade associada às formas graves. Os ratos são considerados os principais reservatórios na transmissão urbana. Entretanto, não existem estudos que sistematicamente definam os fatores de infestação por ratos e as características ambientais que influenciam o risco de infecção por *Leptospira*. **Objetivo**: Determinar a associação entre infestação de roedores e infecção por *Leptospira* em um estudo de coorte prospectiva realizado em uma comunidade carente de Salvador-BA. **Métodos**: De 2004 a 2007, realizamos inquéritos sorológicos anuais em uma coorte de 2.003 habitantes para identificar infecções por *Leptospira*. Identificamos infecções por soroconversão no teste de microaglutinação. Realizamos um estudo de caso-controle aninhado onde definimos como domicílios-casos aqueles que tiveram um ou mais indivíduos infectados. Os controles foram aleatoriamente selecionados dentre os domicílios que tiveram indivíduos sem infecção. Avaliamos por inspeção domiciliar sinais de infestação por roedores e características ambientais. Realizamos regressão logística para identificar fatores de risco para infecção. **Resultados**: Dos 80 domicílios-casos e 109 controles, 78% e 42%, respectivamente, apresentaram fezes e tocas de ratos. Nos 92 domicílios com fezes de roedores, 85% foram de *Rattus norvegicus*, 15% de *Mus musculus* e 5% de *Rattus rattus*. Identificamos como fatores de risco de infecção: fezes de *R. norvegicus* (OR 4.6 IC 95% 1.9-10.7), tocas (OR 2.8, IC 95% 1.1-7.3), parede de domicilio sem reboco (OR 2.5, IC 95% 1.1-7.4) e renda domiciliar per capita (OR 0.9 por US$/dia, IC 95% 0.8-0.9). **Conclusões**: Identificamos alta infestação por *R. norvegicus* na comunidade carente estudada. Esta infestação foi o maior fator preditivo de risco para leptospirose. Medidas de controle precisam ser direcionadas para diminuir a densidade e a proximidade de *R. norvegicus* no ambiente domiciliar. Além disso, identificamos fatores ambientais relacionados com infestação que podem ser usados pelos Centros de Controle de Zoonoses para identificar domicílios de alto risco para leptospirose.

Rat Infestation and Incidence of Leptospirosis Infection in Urban Slums of Brazil

Running head: Rat Infestation and Transmission of Urban Leptospirosis

Federico Costa, Renato Barbosa Reis, Norlan Santos, Guilherme S Ribeiro, Ridalva DM Felzemburgh, Deborah Bittencourt Mothe Fraga, Wildo Araujo, Carlos Santana, Mitermayer G Reis, and Albert I Ko

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brazil (F Costa MSc, R B Reis MSc, N Santos Biol., G S Ribeiro MD PhD, R D M Felzemburgh DN MSc, D B M Fraga PhD, M G Reis MD, PhD, A I Ko MD); Programa de Treinamento em Epidemiologia Aplicada aos Serviços do SUS, Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, Brasil (W. Araujo Med. Vet.); Centro de Controle de Zoonoses, Secretaria Municipal de Saúde, Ministério da Saúde, Salvador, Brazil (C Santana Biol.); and and Yale School of Public Health, Yale University, New Heaven, USA (A I Ko MD).

Correspondence to: Dr. Albert Icksang Ko, Yale, Yale School of Public Health.
60 College Street; P.O. Box 208034; New Haven, United States, CT 06520-8034; e-mail: albert.ko@yale.edu

Keywords:

Leptospirosis, infection, *Rattus norvegicus*, urban slums, epidemiology, risk factors

ABSTRACT

Background: Leptospirosis is a geographically widespread zoonosis. Urban epidemics of leptospirosis occur in cities throughout the developing world where it is transmitted to humans by the rodent *Rattus norvegicus*. To determine the risk of *Leptospira* infection in households in a Brazilian slum area, we analyzed rodent related markers in households. **Methods**: From 2004 to 2007, serologic surveys were performed annually in a cohort of 2003 inhabitants to identify *Leptospira* infections. Microagglutination tests were performed to identify infection. A nested case-control study was conducted in the study site. A household was regarded as a case household if at least one new *Leptospira* infection occurred among cohort subjects. Control households were randomly selected among the population of households with no *Leptospira* infection episode. Households were surveyed for signs of rodent infestation and environmental characteristics. We used conditional logistic regression modeling to identify risk factors for *Leptospira* infection. **Results**: Out of 80 case households and 109 controls, 78% and 42%, respectively, presented rodent feces and burrows. Between 92 households with evidence of rodent feces, 85% were from *Rattus norvegicus*, 15% from *Mus musculus* and 5% from *Rattus rattus*. We identified the following independent risk factors: *R. norvegicus* feces (OR 4.6 CI 95% 1.9-10.7), burrows (OR 2.8, CI 95% 1.1-7.3), unplastered walls (OR 2.5, CI 95% 1.1-7.4) and household per capita income (OR 0.86 for an increase of US$ 1 per day, CI 95% 0.75-0.99). **Conclusions**: We identified high level of *R. norvegicus* infestation. Signs of infestation were the major risk factors for *Leptospira* transmission. Specific control interventions need to be developed for slum areas to decrease density and proximity of *R. norvegicus* to household environments. Additionally, we identified environmental markers related with infestation that may be used by the Zoonotic Control Center to identify households at high risk of leptospirosis transmission.

INTRODUCTION

Worldwide, leptospirosis is a major public health problem caused by spirochetes belonging to the genus *Leptospira* (1-2). *Leptospira* are categorized into more than 200 serovars that are related to particular hosts (3). The primary habitat of the pathogenic spirochetes is the kidney of rodents, which serve as a main reservoir, in addition to other animal species. Reservoirs maintain chronic infection and shed leptospires in their urine which can persist in the environment from weeks to months (2). Leptospirosis is an environmentally-transmitted disease since the major mode of transmission is contact with water or soil contaminated with reservoir urine (1-2). Half million leptospirosis cases are estimated to occur annually worldwide (4), but higher prevalence is reported in tropical areas (2). Leptospirosis has traditionally been a sporadic rural disease associated with occupational risk behaviors (3). In the last decades, epidemics during the raining season in poor communities of large cities have highlighted the emergence of a new epidemiological pattern (5). Poor and over-crowded urban settings (slums) present potentially ideal ecological conditions for leptospirosis transmission due the lack of environmental sanitation that support high reservoir populations (6). The spectrum of human disease caused by leptospirosis is wide, ranging from a self-limiting flu-like illness to severe disease forms such as Weil and pulmonary hemorrhagic syndromes (1-2, 7-8). Nevertheless, the great majority, up to 90%, of leptospiral infections are either subclinical or of mild severity (3, 9).

Exposure to environmental factors, determined by differences in the inoculum size, has been proposed as one of the major reasons for the diverse outcome after infection with *Leptospira* (6, 10-11). However, risk factors have not been extensively studied for the different clinical outcomes. In urban areas, most studies have used ecological and case-control designs to examine risk factors associated with severe leptospirosis, whereas cross-sectional studies have been used to identify asymptomatic *Leptospira* transmission. Ecological (5, 12)

and case-control (10, 13) studies performed in slums located within Brazilian cities have shown that transmission occurs in the household environment. These studies have raised hypotheses that exposures to open sewers, refuse, floodwater and rats may play a role in severe disease. Moreover, in households where severe leptospirosis cases occurred, the vector, *R. norvegicus* had high carriage levels of *L. interrogans* serovar Copenhageni (14). On the other hand cross-sectional studies (6) have identified several environmental risk factors for the presence of *Leptospira* antibodies in healthy subjects, including proximity to open sewer, floodwater, sighting rats and ownership of chicken. Cross-sectional designs which use serologic evidence for a prior *Leptospira* infection as the outcome have limitations in establishing relationships between exposure and disease outcome. Since longitudinal serologic evaluations are required to identify asymptomatic and sub-clinical infections, prospective cohorts are appropriate studies to identify etiologic relationship.

The absence of population-based, prospective epidemiologic data in urban areas has impeded the identification and verification of risk factors. Until the mechanisms of leptospirosis transmission are defined, the natural history of the disease and development of a rational control program remains incomplete. Here, we present the results of a case-control study nested in a prospective, population based cohort in an urban slum area of Brazil. The purpose was to identify environmental-associated risk factors for asymptomatic or subclinical *Leptospira* transmission.

METHODS

Study site and previous studies

The study was conducted in the Pau da Lima (Fig. 1A) community which is situated in the periphery of Salvador, a city of 2.9 million inhabitants (15) in Northeast Brazil. The study site characteristics of the Pau da Lima community have been described elsewhere (6). Briefly, the study site has a high human population density within an area of 0.46 km2, located in four valleys, characterized by a strong deficit in basic sanitation.

During 2003 and 2004, a community-based survey of exposure to *Leptospira* was conducted of 3,171 inhabitants greater than five years of age who were randomly selected from the study area. The overall prevalence of *Leptospira* antibodies was 15·4% (6). This study was used as baseline for a prospective cohort study involving 2003 persons. Cohort subjects were followed in three consecutive serologic/epidemiologic surveys, at annual intervals during the years 2004/5, 2005/6 and 2006/7 (unpublished data).

Case-Control Study

A nested case-control study was conducted in the study site. A household was regarded as a case household if at least one new *Leptospira* infection occurred among cohort subjects that were permanent household members during the 3 years of follow-up. Control households were randomly selected from the same cohort among the population of households that fulfilled three requirements: a) no *Leptospira* infection episode in permanent household members, b) at least one household member with blood collection for each cohort follow-up year, and c) a minimum distance of 30 m to the nearest case household. Because *R. norvegicus* possesses a home range between 30-150 m in urban areas (16-17), the last criteria was implemented in order to avoid overmatching between case and control households with

regard to rodent infestation. Blinding of the case status of the households was possible because two of the authors (R.D.M. and R.B.R), who did not participate in the household rodent survey, prepared the lists and maps of the households, used during rodent survey, without identification about case status.

The sample size for case-control study was 230 households represented by 115 case households with a related control. This size was selected to provide an estimated odds ratio ≥2, power (1-β) 0.8, significance level (α) 0.05, and expected exposure between pairs in the case-control set (φ) 0.2-0.4, calculated using Epi-Info for Windows software (Centers for Disease Control and Prevention, Atlanta, GA).

Ethical clearance for this study was granted by the Ethical Committee in Research of the Oswaldo Cruz Foundation and IRB Committee of Weill Medical College of Cornell University.

Serologic evaluation and definitions of leptospiral infection

The study team collected blood samples from subjects during cohort enrollment and during follow-up visits between October, 2004 and January, 2007. Microagglutination tests (MAT) were performed on sera from the initial and follow-up study visits to determine a baseline presence of antibodies against *Leptospira* and the occurrence of incident infections during follow-up. As previously described (6), a panel of five reference strains and two clinical isolates (5) were used which included *L. interrogans serovars* Autumnalis, Canicola and Copenhageni; *L. borgspetersenii* serovar Ballum, and *L. kirschneri* serovar Grippotyphosa. 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. A positive control serum with a known titer and a negative control were always included in the battery. New infection was defined as seroconversion from a

baseline MAT titer of zero to a follow-up titer ≥1:50. Re-infection was defined as four-fold rise in the follow-up MAT titer in relation to a baseline titer ≥1:25.

Household rodent survey

Surveys of case and control households were conducted by the authors (F.C. and N.S.) and experienced rodent control specialists of the Zoonosis Control Center from Salvador. Household surveys were performed between October and November of 2007. The survey team used a modified exterior inspection form, adapted from the CDC manual (18). The form consisted of the following eight groups of variables: 1) 3 questions on demographic information, 2) 4 variables on premise type; 3) 4 variables on food sources for rodents; 4) 2 variables on water sources for rodents; 5) 11 variables on harborage for rodents; 6) 5 variables on entry/access for rodents; 7) 6 variables on signs of rodent infestation; and 8) 3 questions on domestic animals (Figure 2). Because of the environmental and socioeconomic differences found in Salvador some variables from CDC manual needed to be excluded or modified and additional variables incorporated (details are shown in Supplemental table 1).

The maximum time lag between occurrence of *Leptospira* infection and assessment of household rodent survey was three years. To minimize temporal effect, during survey we asked the head-of-household if domicile structure, peridomicile, open sewer or refuse deposit changed from the date of *Leptospira* infection. Fictitious *Leptospira* infection dates were created for control households to preserve the blinded status of the study.

Because the proportion of cases and control households surveyed did not represent the general proportion of those households in Pau da Lima we estimated a general infestation rate using the following equation: General infestation rate = ((proportion of infested case household * number of case households in Pau da Lima) + (proportion of infested control

household * number of control households in Pau da Lima))/ Total number of households in Pau da Lima.

Geographical information System (GIS) survey

During September 2007 GIS surveys were done to measure environmental variables. Following methodology previously described (6), the study site was surveyed to record the location and size of sites of open refuse deposits, open sewage and rainwater drainage systems (Fig. 1B). These data were used to obtain the distances from each studied household to the nearest drainage systems, open refuse deposit and the lowest point in the valley

Statistical analysis

Epidemiological and laboratory data were double-entered and validated using the Epi-Info for Windows software (Centers for Disease Control and Prevention, Atlanta, GA). Data for individual subjects were linked by location of residence to spatially-coded information for households and environmental attributes within the study site. Chi-square and Wilcoxon rank sum tests were used to compare categorical and continuous data, respectively, in the bivariate analysis to investigate the association of case and control status with regard to different environmental household characteristics. A p-value of 0,05 or less in two-sided testing was used as criteria for a statistically significant difference. Concordance correlation coefficient between environmental variables was analyzed using the kappa index test. A kappa value of 0 to 0.20 was considered poor, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 good, and 0.81 to 1.00 excellent.

Variables with $p<0.1$ in the univariate analysis were included in a multivariate conditional logistic regression analysis. Four models were used to fit the different blocks of variables to the binary outcome of case and control households. The first model, included rodent infestation variables. The second model, included environmental variables that we

hypothesized were related to rodent infestation or to *Leptospira* infection. The third model included socioeconomic status and household variables. The fourth and final model included variables retained from the first, second and third multivariate models. This staged approach is an accepted method of variable selection (19-20). A backward elimination strategy was used to obtain models using SAS program for Windows software (21).

To test the stability of our final model, we tried alternate methods to determine whether the resultant model would differ from our original final model. First, we used forward selection technique instead of backward selection. Second, all variables with $p<0.1$ in the univariate analysis were included in a unique multivariate conditional logistic regression analysis. To compare the goodness of fit, Akaike Information Criteria values were calculated for separate models (22).

RESULTS

During the three years of the cohort follow-up, 85 case households were registered. Sixty-two case households (73%) contained one infected subject, 18 households (22%) two infected subjects, and four household (5%) three infected subjects. Twenty-eight case households (33%) were identified in 2005, 26 (30%) in 2006 and 31 (38%) in 2007. Highest titers were directed against *L. interrogans* serovar Copenhageni in subjects of 81 (95%) case households. Of the 186 households that fulfilled criteria to be a control household, 115 were randomly selected for evaluation (a flowchart showing case and control household selection is provided in Additional figure I). Rodent survey was performed in 189 (95%) households (80 case and 109 control households). Of eleven households (5 case and 6 control) that could not be inspected, ten were closed during at least three visits and one case household was found destroyed. The spatial distribution of surveyed case and control households in the study area is shown in figure 1C and 1D respectively.

Based on the survey, 78% of the cases and 42% of control households presented at least one rodent sign and were considered infested. We found rodent infested case and control households distributed trough all the study area (figures 1C and 1D). Considering the total number of cases (N = 85) and control households (N = 599) the general infestation for the study area was estimated in 45.9%. Major active rodent signs were rodent burrows, runs and *R. norvegicus* fecal droppings presenting in 84 (42%), 84 (42%) and 78 (39%) of the households respectively (Table 1). Most of the rat burrows and runs apparently belonged to *R. norvegicus* because their connection with *R. norvegicus* fecal droppings, additionally *R. norvegicus* fecal droppings presented good concordance with rodent burrows (kappa=0.61) and moderate concordance with rodent runs (kappa=0.51). Of the 101 households with fecal

droppings, 73% presented signs of *R. norvegicus*, 14% of *M. musculus* and 4% of *R. rattus*, additionally 9% households presented fecal droppings from non identified species.

Eighty (42%) households, 35 cases and 45 controls, presented modifications between the data of *Leptospira* infection and data of exposure survey. Modifications were registered in domicile structure (n=68), peridomicile (n=44), open sewer (n=11) and refuse deposit (n=4), 36%, 23%, 6% and 2% of the total of households. Case households presenting modifications (n=35) were compared with case households which did not present modifications (n=45). The same analysis was performed for control households. Risk factors analysis did not show significant differences in proportions between groups for variables included in the final model (Supplemental tables 2A and 2B) and all 189 households were considered for further analyses.

In bivariate analyses, household environmental variables were found to be associated with case households: exposed garbage, other food and plants, fruit trees, open stores of human food, access to water (including standing water), lumber/clutter on ground, large rubbish, dilapidated fences and walls, plant-related debris(including bushes or shrubbery), presence of exposed earth, household built on earthen slope, structural deficiencies, holes in the roof and un-plastered walls. A larger percentage of case household ($P<0.01$) showed signs of rodent infestation related to *R. norvegicus*, such fecal droppings, burrows and runs. Significant associations were not found for domestic animals (Table 1). Additionally, the risk of acquiring *Leptospira* infection in a household was associated with indicators of low socioeconomic status as per capita income and number of inhabitants in the house.

Multivariable logistic regression results are shown in Table 2. Two of three variables included in the first model of rodent infestation factors were significant. Presence of *R. norvegicus* fecal droppings and rodent burrows was more likely in case households. The second model including household environment variables retained three significant risk

factors, access to water, domicile built on slope and un-plastered exterior wall surface. In the third model that included socioeconomic status of household only the per capita income variable remained significant.

The final model (fourth model) retained five variables: two variables from the model of rodent infestation factors, two from the model of household environmental variables and other one from socioeconomic status model. *R. norvegicus* fecal droppings had the strongest association with case households in the final model (OR = 4.57, (95% CI = 1.95-10.67). Rodent burrows (OR = 2.80, 95% IC = 1.06-7.36), access to water (OR = 2.73, 95% IC = 1.24-6.04) and un-plastered exterior wall surface (OR = 2.48, 95% IC = 1.10-7.36) were additional independent risk factors. Household per capita income (OR 0.86 for an increase of US$1.00 per day, 95% CI 0.75–0.99) was a protective factor for acquiring *Leptospira* infection in a household. A significant environment variable included in the second model, but excluded from the final model, was domicile built on slope (Table 2).

An additional final model (fifth model) was created replacing the variables *R. norvegicus* fecal droppings, rodent burrows and runs for the combined variable of any sign of rat infestation (Table 2). With the exception of un-plastered exterior wall surface this model retained similar variables as the previous model, but showed a poorer fitness. It includes the variables of rodent active signs (OR = 3.75, 95% CI = 1.88-7.48), access to water (OR = 2.19, 955 CI = 1.07-4.48) and per capita income (OR = 0.82, 95% CI = 0.72-0.95).

DISCUSSION

Few other studies have attempted to define the household conditions associated with peridomestic transmission of *Leptospira*, and none have incorporated both, systematic rodent infestation evaluation and prospective laboratory confirmation of infection in their epidemiologic design**.** We have shown that in a slum settlement in Brazil, after adjustment for confounders, specific markers of rodent infestation are strongly associated with the occurrence of *Leptospira* infection in a household. Most of the case houses (78%) had signs of rodents, whereas only 42% of control houses had those signs. The absence of rodent signs indicates that the house is less likely to have *Leptospira* transmission. Similar risk factors were described for Lassa fever in refugee camps in Sierra Leone (23) and for hantavirus pulmonary syndrome in the southwestern United States (24). Therefore, in areas with high risk for *Leptospira* infection, rodent management programs could increase their cost-effectiveness by prioritizing interventions in households with presence of rodent-related markers.

Our results confirm the previous findings and further support the role of peridomestic transmission of *Leptospira* in the slum populations of developing countries. Several studies of urban leptospirosis found that environmental attributes of slum households are risk factors for acquiring *Leptospira* antibodies and severe leptospirosis (5-6, 10, 12-13, 25-26). Additionally, high concentrations of *L. interrogans* serovar Icterohaemorrhagiae from sewage and river water were identified in urban slums of Peru (11). We did not evaluate infection risk at the workplace, a possible confounding factor, in this study. However, in a previous report, work related exposures were not independent risk factor for presence of *Leptospira* antibodies (6). Still those factors could contribute to infection in this population. A case-control study found that workplace-related exposures to contaminated environment are a risk factor for severe leptospirosis in addition to attributes associated with the household environment (10).

The results of our household surveys indicate that rats were widespread in the study area of Pau da Lima during the sampling period in 2007. A high degree of infestation, reaching almost 50% of the households, is not surprising in the light of the absence of any significant rodent control measures in Pau da Lima until the survey period. The proportion of rodent infested households in this study was similar to those reported in studies on poor urban areas in Latin America, where infestation rates varied between 40 to 70% (27-30). These rodent infestation rates are higher than those encountered in developed countries (31-33). The Center for Disease Control and Prevention (CDC – USA) considers that residents of buildings with infestation rates equal or above 26% are at high risk for rodent borne diseases (18). *Rattus norvegicus* was the dominant species in the urban ecosystem of Salvador, brown rats feces represented 80% of the feces signs in our study. Predominance of *R. norvegicus* has been previously reported in other poor urban areas in Latin American (29-30, 34), although *R. rattus* was most frequent in neighborhoods of Sao Paulo (Brazil) (28) and Tumero (Venezuela) (27). In North American (31, 33) and European cities (35-37) the most common rat is *R. norvegicus*. We found that presence of *R. norvegicus* fecal droppings and rat burrows were independent risk factor for *Leptospira* transmission in the household environment. In a study performed in 1998, *R. norvegicus* populations showed a high level of *Leptospira* serovar Copenhagen carriage in households where leptospirosis cases resided (14). The presence of a highly frequent predominant reservoir, in urban areas at risk of leptospirosis transmission, highlights the importance of *R. norvegicus* control.

We used a large set of environmental variables to identify potential sources of *Leptospira* infection and confounders related to rodent reservoir infestation. Presence of un-plastered wall in the home was significantly associated with case households, even when adjusted for signs of rat infestation and income per capita. The presence of un-plastered wall may be a proxy for socioeconomic status not captured by the income variable or an indicator

for behavior care of the household. We believe it is less probable that un-plastered walls could be a risk factor for rodent household access, because *R. norvegicus* prefers outdoor environments (18). Two variables, domicile built on slope and standing water, were not retained in the final model. Domiciles built on an incline provide suitable space for rodent harborage, and selection of slopes to build burrows by *R. norvegicus* was registered in a previous study (38). Standing water (variable which includes water from open sewer) could be a risk factor for *Leptospira* infection. Presence of open sewer in the household was risk factor for *Leptospira* antibodies and severe leptospirosis (6, 10). Additionally, standing water, especially sewers, can provide the water source for *R. norvegicus* and therefore may be related to rodent infestation (28, 39-40).

The socioeconomic variable per capita income was an independent risk factor for *Leptospira* infection in a household. Low income is considered an indicator of health inequality in Brazil (41) and has been associated to risk of *Leptospira* transmission in the study area previously (6). This factor may relate to risky behaviors, such as cleaning open sewers after flooding events, limited use of protective clothing to reduce the risk of entry of the *Leptospira* spirochete, or poor household hygiene providing rodent causative conditions.

The study was limited by the time lag between occurrence of *Leptospira* infection and assessment of housing rodent survey. We shown that four major causes of modification in a household did not influence in the studied variables, but other not registered environmental factors could be changed during the study period. **(2)** Because the control-household definition -mainly the criteria of distance of 30 m between cases and control households- only 186 (32%) from 569 potential control households were available to randomization. A reduced number of control-households could be affected the effect of the randomization in the selection of control-households. Additionally, the distance of 30 m between cases and control households may have inadvertently masked other risk factors. **(3)** Rodent management

programs aim to reduce the incidence of severe leptospirosis cases, this study presents information about risk in households with *Leptospira* infection. It is not defined whether risk factors for *Leptospira* infection are the same for severe leptospirosis. Nevertheless studies indicate that subjects with asymptomatic infection and severe leptospirosis may share exposures in the environment where they reside. A previous study showed that members of households living with an index case of leptospirosis had higher risk of having serologic evidence for a prior infection than members of households in the same communities (13). Additionally, environmental deficiencies such as the presence of an open sewer near to the household and sighting rats in the peridomicile were independent risk factors for both severe leptospirosis (10) and previous *Leptospira* infection (6). **(4)** Finally, our results may not be generalizable to other settings; however 67% of the population of Salvador lives in similar social and environmental conditions than the inhabitants of Pau da Lima (42).

In conclusion, we identified high level of *R. norvegicus* infestation. Signs of this infestation were the major risk factors for *Leptospira* transmission. Specific control interventions for slum areas need to be developed to decrease density and proximity of *R. norvegicus* to households environments. Additionally, we identified environmental markers related with infestation that may be used by the Zoonotic Control Centers to identify households at high risk of leptospirosis transmission.

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CONFLICT OF INTEREST

We declare that none of the authors have any potential conflict of interest.

FIRST AUTHOR"S BIOGRAPHICAL SKETCH

MSc Federico Costa is a biologist and PhD student at the Oswaldo Cruz Foundation, Brazilian Ministry of Health in Salvador, Brazil, whose research interests focus on infectious disease problems and reservoirs ecology that affect urban slum populations.

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Table 1: Rodent-related and environmental risk factors for *Leptospira* infection among 80 case and 109 control households at the community study site, Salvador, Brazil

Household characteristics	**Case***	**Control***	
	(*n* = 80)	**(*n* = 109)**	
	No. (%) or median (IQR)†		***P*‡**
Demographics			
No. of inhabitants	4 (4-6)	4 (3-5)	<0.05
Male sex	2 (1-2)	1 (1-2)	-
Per capita income, US$/d	2.6 (1.5-3.8)	3.7 (2.4-5.5)	<0.01
Squatter household§	73 (91)	94 (86)	-
Premise type and details¶			
Residential use only¶	79 (99)	109 (100)	-
Borders on a vacant lot	10 (12)	12 (11)	-
Open sewer <10m distance	23 (21)	22 (27)	-
Distance from open sewer, m	23.2 (12.4-44.7)	21.4 (7.9-36.2)	-
Distance from open refuse deposit, m	74.4 (49.1-105.1)	65.1 (45.6-83.6)	-
Level above lowest point in valley, m	20.5 (10.5-30.2)	21.0 (13.6-34.9)	-
Borders on an abandoned house	13 (16)	12 (11)	-
Access to food sources¶			
Exposed garbage¶	45 (56)	44 (40)	<0.05
Animal food¶	22 (27)	27 (25)	-
Other food & plants¶	32 (40)	30 (27)	<0.1
Fruit trees	45 (56)	38 (35)	<0.01
Open stores of human food	19 (24)	13 (12)	<0.05
Access to water¶	33 (41)	20 (18)	<0.01

Standing water¶	24 (30)	15 (13)	<0.05
Leaks¶	9 (11)	5 (5)	-
Harborage for rodents¶			
Abandoned vehicles¶	1 (1)	0 (0)	-
Abandoned appliances¶	48 (60)	68 (62)	-
Lumber/clutter on ground¶	58 (72)	66 (61)	<0.1
Other large rubbish¶	51 (64)	51 (47)	<0.05
Outbuildings/Privies¶	18 (22)	15 (14)	-
Dilapidated fences & walls¶	24 (30)	21 (19)	<0.1
Plant-related¶	70 (87)	84 (77)	<0.1
Bushes or shrubbery¶	40 (50)	29 (26)	<0.01
Ornamental plants¶	56 (70)	68 (62)	-
Presence of exposed earth	64 (80)	61 (56)	<0.01
Built on earthen slope#	54 (67)	44 (40)	<0.01
Entry/Access¶			
Structural deficiencies¶	54 (67)	53 (49)	<0.01
Hole(s) in roof	50 (62)	49 (45)	<0.05
Hole(s) in wall	12 (15)	17 (15)	-
Hole(s) in floor	12 (15)	11 (10)	-
Un-plastered walls**	66 (82)	63 (57)	<0.01
Rodent active signs¶			
Active signs¶	63 (78)	46 (42)	<0.01
Rodent burrows	52 (65)	32 (29)	<0.01
Rodent runs	46 (57)	38 (35)	<0.01
R. norvegicus feces	53 (66)	25 (23)	<0.01

R. rattus feces	3 (4)	1 (1)	-
M. musculus feces	4 (5)	11 (10)	-
Domestic animals			
Dogs	38 (47)	40 (36)	-
Cats	8 (10)	15 (14)	-
Chickens	8 (10)	7 (6)	-

* Case and control households comprised of households in wich cohort subject(s) with evidence of *Leptospira* infection resided and neighborhood households which were located >30m of a case household and did not have a member with evidence of *Leptospira* infection during the study period, respectively.

† Median and inter-quartile range (IQR) values are shown for continuous variables.

‡ Values are not shown for non-significant associations.

§ Household constructed in a space that the squatter does not own, rent or otherwise have permission to use.

¶ Categories and variable defined in the CDC form (43).

Presence of exposed earth slope (>45º) within 10m of the household.

**Walls composed of exposed bricks without external application of stucco or plastering.

Table 2: Logistic regression analysis of rodent-related and environmental risk factors for *Leptospira* infection

Variables	**OR (95% CI)†**	
	Unadjusted‡	**Adjusted§**
1st model: rodent infestation variables		
Rodent burrows	4.46 (2.41-8.28)	2.49 (1.03-6.24)
Rodent runs	2.52 (1.39-4.57)	-
R. norvegicus feces	6.59 (3.46-12.55)	5.02 (2.24-11.25)
2nd model: Household environment variables		
Exposed garbage¶	1.89 (1.05-3.40)	-
Other food & plants¶	1.75 (0.95-3.24)	-
Fruit trees	2.40 (1.33-4.34)	-
Open stores of human food	2.30 (1.05-4.99)	-
Access to water¶	3.12 (1.61-6.03)	2.35 (1.17-4.71)
Standing water¶	2.68 (1.30-5.54)	-
Lumber/clutter on ground¶	1.71 (0.92-3.20)	-
Other large rubbish¶	2.00 (1.10-3.61)	-
Dilapidated fences & walls¶	1.79 (0.91-3.52)	-
Plant-related¶	2.08 (0.93-4.63)	-
Bushes or shrubbery	2.75 (1.49-5.07)	-
Presence of exposed earth	3.14 (1.61-6.12)	-
Built on earthen slope#	3.06 (1.67-5.61)	2.13 (1.11-4.07)
Structural deficiencies¶	2.19 (1.24-3.99)	-
Hole(s) in roof	2.04 (1.13-3.67)	-
Un-plastered walls**	3.44 (1.72-6.86)	2.52 (1.21-5.24)

3rd model: Socioeconomic status of household variables		
Per capita income, US$/d	0.78 (0.68-0.90)	0.78 (0.68-0.90)
No. of inhabitants	1.10 (0.96-1.27)	-
4th model: Significant variables from each block/model		
Rodent burrows	4.46 (2.41-8.28)	2.80 (1.06-7.36)
R. norvegicus feces	6.59 (3.46-12.55)	4.57 (1.95-10.67)
Access to water¶	3.12 (1.61-6.03)	2.73 (1.24-6.04)
Built on earthen slope#	3.06 (1.67-5.61)	-
Un-plastered walls**	3.44 (1.72-6.86)	2.48 (1.10-7.36)
Per capita income, US$/d	0.78 (0.68-0.90)	0.86 (0.75-0.99)
5th model: Significant variables from each block/model replacing Rodent active signs for Rodent burrows and *R. norvegicus* feces		
Rodent active signs¶	5.07 (2.63-9.78)	3.75 (1.88-7.48)
Access to water¶	3.12 (1.61-6.03)	2.19 (1.07-4.48)
Built on earthen slope#	3.06 (1.67-5.61)	-
Un-plastered walls**	3.44 (1.72-6.86)	-
Per capita income, US$/d	0.78 (0.68-0.90)	0.82 (0.72-0.95)

* Case and control households comprised of households in which cohort subject(s) with evidence of *Leptospira* infection resided and neighborhood households which were located >30m of a case household and did not have a member with evidence of *Leptospira* infection during the study period, respectively.

† Odds ratios (OR) and 95% confidence intervals (CI).

‡ Unadjusted OR are shown for variables which were significant ($P < 0.05$) in the univariate analyses.

§Adjusted OR are shown for variables which were significant ($P < 0.05$) in the backward logistic regression analysis.

¶ Categories and variable defined in the CDC form (Centers for Disease Control and Prevention, 2006).

Presence of exposed earth slope (>45º) within 10m of the household.

** Walls composed of exposed bricks without external application of stucco or plastering.

Figure Legends

Figure 1. Slum community site in the city of Salvador, Brazil according to spatial distribution of case and control households and environmental attributes of the community site. (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) Topographic map generated by the digital terrain model and distribution of open sewage drainage systems. (C) Spatial distribution of rat infested case (red solid circles) and non-infested (red open circles) households. (D) Rat infested control households are represented in solid blue circles and non-infested control households in open blue circles.

Figure 2. Environmental variables related to source of food, water, harborage and access for rodents and rodent active signs in the study area. (A and B) Photographs of the typical environment at the community study site, which shows a valley in which households are situated and the proximity of households to open sewers, exposed garbage and bushes or shrubbery. (C) Exposed garbage. (D) Water leaks. (E) Structural deficiencies. (F, I and K) Rodent burrows. (G and J) *Rattus norvegicus* fecal droppings. (H and K) Rodent runs.

Figure 1:

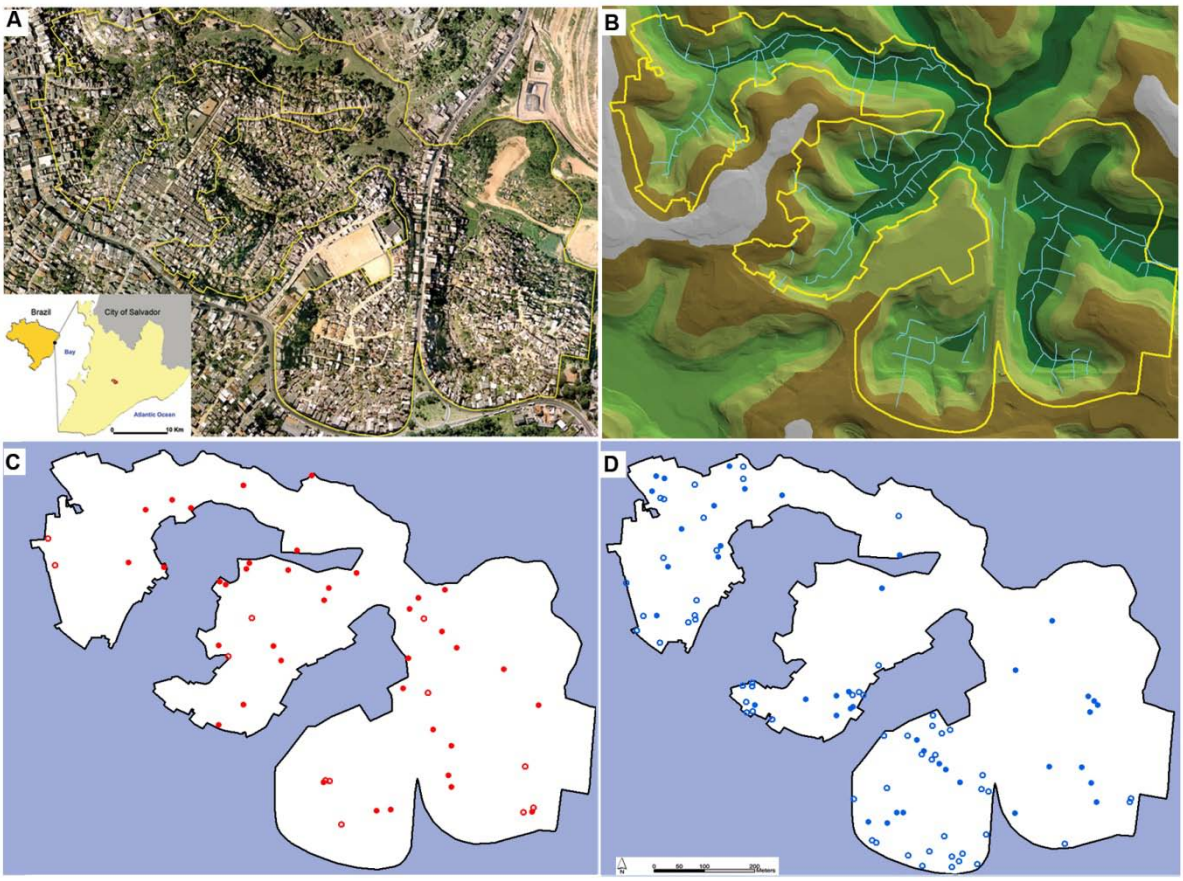


Figure 2

Supplemental table 1. Description of variables included in the modified exterior inspection form, adapted from the CDC manual.

Variables	CDC Survey form No.*	Comments
Demographics		
Number of inhabitants	-	New
Proportion of males	-	New
Per capita income, US$/d	-	New
Premise type and details		
Residential use only	1	Included
Commercial & residential	2	Included
Commercial	3	Not included
Vacant lot	4	Modified: Borders on a vacant lot
Food comercial establishment	5	Not included
Vacant	6	Not included
No. of dwelling units	7	Not included
Sewer on premise	8	Modified: Open sewer <10m
Borders an abandoned house	-	New
Food sources for rodents		
Unapproved refuse storage	9	Not included
Exposed garbage	10	Included
Animal food	11	Included
Other food & plants	12	Included
Fruit trees	-	New
Open stores of human food	-	New

Water sources for rodents		
Standing water	13	Included
Condensate	14	Not included
Leaks	15	Included
Harborage for rodents		
Abandoned vehicles	16	Included
Abandoned appliances	17	Included
Lumber/clutter on ground	18	Included
Other large rubbish	19	Included
Outbuildings /privies	20	Included
Dilapidated fences & walls	21	Included
Plant related	22	Included
Bushes or shrubbery	-	New
Ornamental plants	-	New
Presence of exposed earth	-	New
Built on earthen slope†	-	New
Entry/Access of rodents		
Structural deficiencies	23	Included
Pipe/wiring gaps	24	Not included
Hole(s) in roof	-	New
Hole(s) in wall	-	New
Hole(s) in floor	-	New
Unplastered walls‡	-	New
Signs of rodent infestation		
Active signs	25	Included

Rodent burrows	-	New
Rodent runs	-	New
R. norvegicus feces	-	New
R. rattus feces	-	New
M. musculus feces	-	New
Domestic animals		New
Dogs	-	New
Cats	-	New
Chickens	-	New

* Variable number in the CDC form (43).

† Presence of uncovered terrain slope, with angle >45º, localized to a ≤ 10m of the household.

‡ Household walls without stucco application in the exterior surface and where is possible to observe the wall bricks.

Supplemental table 2A: Characteristics of case households with and without modifications in domicile structure, peridomicile, open sewer or refuse deposit.

*Case Household characteristics	Modifications in structure, peridomicile, open sewer or refuse deposit		
	Yes (*n* = 35)	**No (*n* = 45)**	
	No. (%) or median (IQR)†		***P*‡**
Demographics			
No. of inhabitants	5 (4-7)	4 (4-6)	-
Male sex	2 (1-2)	1 (1-2)	-
Per capita income, US$/d	2.3 (1.4-3.7)	2.8 (1.5-3.9)	-
Squatter household§	100 (100)	38 (84)	0.014
Premise type and details¶			
Residential use only¶	35 (100)	44 (98)	-
Borders on a vacant lot	6 (17)	4 (9)	-
Open sewer <10m distance	7 (20)	15 (33)	-
Distance from open sewer, m	20.9 (10.6-34.1)	25.7 (10.6-34.1)	-
Distance from open refuse deposit, m	69.5 (39.8-98.2)	75.0 (58.5-110.0)	-
Level above lowest point in valley, m	18.8 (11.2-31.8)	21.3 (9.8-29.6)	-
Borders on an abandoned house	7 (20)	6 (13)	-
Access to food sources¶			
Exposed garbage¶	23 (66)	22 (49)	-
Animal food¶	12 (34)	10 (22)	-
Other food & plants¶	15 (43)	17 (38)	-
Fruit trees	24 (68)	21 (47)	-

Open stores of human food	9 (26)	10 (22)	-
Access to water¶	15 (43)	18 (40)	-
Standing water¶	14 (40)	10 (22)	-
Leaks¶	5 (14)	4 (9)	-
Harborage for rodents¶			
Abandoned vehicles¶	1 (3)	0 (0)	-
Abandoned appliances¶	24 (68)	27 (60)	-
Lumber/clutter on ground¶	27 (77)	31 (69)	-
Other large rubbish¶	21 (60)	27 (60)	-
Outbuildings/Privies¶	7 (20)	10 (22)	-
Dilapidated fences & walls¶	11 (31)	13 (29)	-
Plant-related¶	33 (94)	34 (75)	0.02
Bushes or shrubbery¶	22 (63)	18 (40)	0.04
Ornamental plants¶	26 (74)	30 (66)	-
Presence of exposed earth	30 (85)	34 (75)	-
Built on earthen slope#	27 (77)	27 (60)	-
Entry/Access¶			
Structural deficiencies¶	34 (97)	42 (93)	-
Hole(s) in roof	24 (68)	26 (58)	-
Hole(s) in wall	6 (17)	6 (13)	-
Hole(s) in floor	3 (8)	9 (20)	-
Un-plastered walls**	31 (88)	35 (79)	-
Rodent active signs¶			
Active signs¶	31 (88)	32 (71)	-
Rodent burrows	26 (74)	26 (58)	-

Rodent runs	23 (65)	23 (51)	-
R. norvegicus feces	26 (74)	27 (60)	-
R. rattus feces	1 (3)	2 (4)	-
M. musculus feces	3 (8)	1 (2)	-
Domestic animals			
Dogs	19 (54)	19 (42)	-
Cats	5 (14)	3 (6)	-
Chickens	5 (14)	3 (6)	-

* Case households in which cohort subject(s) with evidence of *Leptospira* infection resided during the study period.

† Median and inter-quartile range (IQR) values are shown for continuous variables.

‡ Values are not shown for non-significant associations in matched analyses.

§ Household constructed in a space that the squatter does not own, rent or otherwise have permission to use.

¶ Categories and variable defined in the CDC form (43).

#Presence of exposed earth slope (>45º) within 10m of the household.

**Walls composed of exposed bricks without external application of stucco or plastering.

Supplemental table 2B: Characteristics of control households with and without modifications in domicile structure, peridomicile, open sewer or refuse deposit.

*Control Household characteristics	Modifications in structure, peridomicile, open sewer or refuse deposit		
	Yes (*n* = 45)	**No (*n* = 64)**	
	No. (%) or median (IQR)†		***P*‡**
Demographics			
No. of inhabitants	4 (3-5)	4 (3-5)	-
Male sex	2 (1-2)	1 (1-2)	-
Per capita income, US$/d	3.7 (2.3-5.3)	3.7 (2.5-6.1)	-
Squatter household§	37 (82)	57 (89)	-
Premise type and details¶			
Residential use only¶	45 (100)	64 (100)	-
Borders on a vacant lot	2 (4)	10 (15)	-
Open sewer <10m distance	7 (16)	16 (25)	-
Distance from open sewer, m	27.9 (19.3-44.7)	20.3 (10.2-44.9)	-
Distance from open refuse deposit, m	62.6 (39.7-83.6)	70.7 (48.7-84.3)	-
Level above lowest point in valley, m	22.9 (17.2-36.7)	19.1 (10.9-34.1)	-
Borders on an abandoned house	4 (9)	8 (12)	-
Access to food sources¶			
Exposed garbage¶	21 (46)	23 (36)	-
Animal food¶	13 (29)	14 (22)	-
Other food & plants¶	14 (31)	16 (25)	-
Fruit trees	18 (40)	20 (31)	-

Open stores of human food	6 (13)	7 (11)	-
Access to water¶	10 (22)	10 (15)	-
Standing water¶	7 (16)	8 (12)	-
Leaks¶	3 (7)	2 (3)	-
Harborage for rodents¶			
Abandoned vehicles¶	0 (0)	0 (0)	-
Abandoned appliances¶	23 (51)	28 (44)	-
Lumber/clutter on ground¶	31 (69)	35 (55)	-
Other large rubbish¶	33 (73)	35 (55)	-
Outbuildings/Privies¶	2 (4)	13 (20)	0.02
Dilapidated fences & walls¶	10 (22)	11 (17)	-
Plant-related¶	33 (73)	48 (75)	-
Bushes or shrubbery¶	15 (33)	14 (22)	-
Ornamental plants¶	27 (60)	41 (64)	-
Presence of exposed earth	27 (60)	34 (53)	-
Built on earthen slope#	22 (49)	22 (34)	-
Entry/Access¶			
Structural deficiencies¶	42 (93)	62 (97)	-
Hole(s) in roof	23 (51)	26 (41)	-
Hole(s) in wall	8 (18)	9 (14)	-
Hole(s) in floor	5 (11)	6 (9)	-
Un-plastered walls**	29 (64)	34 (53)	-
Rodent active signs¶			
Active signs¶	23 (51)	23 (36)	-
Rodent burrows	13 (29)	19 (29)	-

Rodent runs	21 (47)	17 (26)	-
R. norvegicus feces	8 (18)	17 (26)	-
R. rattus feces	1 (2)	0 (0)	-
M. musculus feces	6 (13)	5 (8)	-
Domestic animals			
Dogs	17 (38)	23 (36)	-
Cats	7 (16)	8 (12)	-
Chickens	3 (7)	4 (6)	-

* Control households comprised households which were located >30m of a case household and did not have a member with evidence of *Leptospira* infection during the study period.

† Median and inter-quartile range (IQR) values are shown for continuous variables.

‡ Values are not shown for non-significant associations in matched analyses.

§ Household constructed in a space that the squatter does not own, rent or otherwise have permission to use.

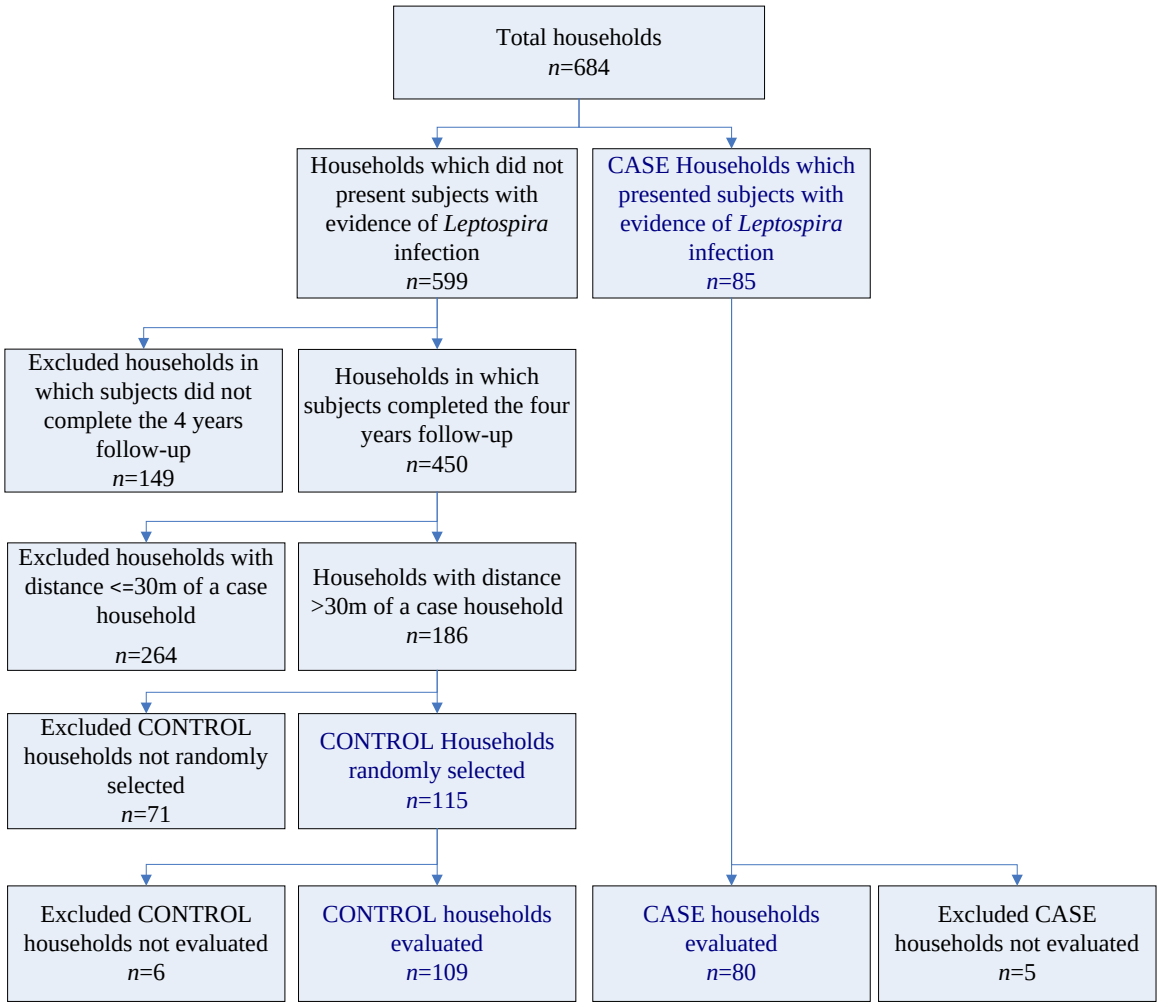
¶ Categories and variable defined in the CDC form (43).

#Presence of exposed earth slope (>45º) within 10m of the household.

**Walls composed of exposed bricks without external application of stucco or plastering.

Additional figure

Supplemental figure 1: Schematic flow diagram of selected case and control households.



4. MANUSCRITO 2

COSTA, F.; RIBEIRO, G. S.; SANTOS, N.; REIS, R. B.; FELZEMBURGH, R. D. M.; BETANCURT, D.; SANTANA, C.; BRANT, J.; REIS, M. G.; KO, A. I. Household rat infestation in urban slum populations: development and validation of a predictive score for leptospirosis. (Artigo a ser submetido).

RESUMO:

Introdução: A leptospirose é uma zoonose de elevada mortalidade, que afeta comunidades urbanas carentes, e os ratos são considerados os principais reservatórios. Entretanto, não existem estudos que definam sistematicamente fatores de infestação por ratos e avaliem sua utilização para predizer o risco de leptospirose. **Objetivos**: Desenvolver e validar um escore domiciliar baseado em características da infestação de ratos para predizer o risco de leptospirose em comunidades carentes de Salvador-BA. **Métodos**: Desenvolvemos um estudo caso-controle 1:2, onde domicílios casos foram aqueles nos quais se registraram casos de leptospirose, identificados entre 2007-2009 em Salvador. Domicílios controles foram localizados a 35-50m do caso. Avaliamos domicílios, registrando sinais de infestação e características ambientais. Domicílios dos anos de 2007-08 (grupo desenvolvimento) foram analisados por regressão logística condicional, a fim de identificar fatores de risco para leptospirose e desenvolver um escore preditivo. Validamos o escore nos domicílios do ano de 2009 (grupo validação) e utilizamos curvas características de recepção (ROC) para analisar o desempenho preditivo do escore. **Resultados**: Identificamos sinais de infestação em 63% (60/95) e 35% (64/184) dos domicílios casos e controles respectivamente. Fatores de risco independentes para leptospirose foram tocas (OR, 3,30; 95% IC, 1,50-7,26), fezes de *Rattus norvegicus* (2,86; 1,24-6,59), trilhas (2,57; 1,06-6,22), casa abandonada <10m (2,48; 1,04-6,02) e domicílio sem reboco (2,22; 1,02-6,02). Designamos valores de escore para cada fator de risco (3, 3, 2, 2 e 2 respectivamente). A área sob a curva ROC foi 0,70 (IC95%, 0,64-0,76) para o grupo de desenvolvimento e 0,71 (95; 0,65-0,79) para o de validação. **Conclusões**: Achamos uma elevada proporção (>44%) de domicílios infestados com *R. norvegicus*. Identificamos e validamos um escore preditivo que identifica domicílios de elevado risco dentro de comunidades com transmissão endêmica de leptospirose. Estes achados sugerem que a triagem da infestação por roedores e a identificação de domicílios de risco podem ser estratégias para dirigir intervenções de controle de roedores em populações de risco.

Household Rat Infestation in Urban Slum Populations: Development and Validation of a Predictive Score for Leptospirosis

Running head: Household rat infestation as a predictor of leptospirosis

Federico Costa, Guilherme S Ribeiro, Norlan Santos, Renato Barbosa Reis, Ridalva DM Felzemburgh, Deborah Betancourt, Carlos Santana, Jonas Brant, Mitermayer G Reis, and Albert I Ko

Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brazil (F Costa MSc, , G S Ribeiro MD, PhD, N. Santos Biol, R B Reis MSc R D M Felzemburgh DN MSc, D Betancourt MSc, , M G Reis MD, PhD, A I Ko MD); Centro de Controle de Zoonoses, Ministério da Saúde, Salvador, Brazil (Carlos Santana Biol.); Programa de Treinamento em Epidemiologia Aplicada aos Serviços do SUS, Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, Brazil (Jonas Brant MSc.); and Yale School of Public Health, Yale University, New Heaven, USA (A I Ko MD)

Correspondence to: Dr. Albert Icksang Ko, Yale School of Public Health, Yale University. 60 College Street; P.O. Box 208034; New Haven, United States, CT 06520-8034; e-mail: albert.ko@yale.edu

Keywords:

Leptospirosis, predictive score, *Rattus norvegicus*, urban slums, epidemiology

ABSTRACT

Background: The domestic rat is believed to be the principal reservoir for urban leptospirosis. However, few if any studies have identified markers for rodent infestation in slum environments and evaluated their use in predicting the risk for leptospirosis.

Methods: Households of leptospirosis cases identified between 2007 and 2009 in Salvador, Brazil and neighboring control households in the same slum communities were enrolled in a case control study. Households were surveyed for signs of rodent infestation and environmental characteristics. We used conditional logistic regression modeling to identify risk factors and develop a predictive score for leptospirosis with data collected from 2007 to 2008. We used receiver operating characteristic (ROC) curve analysis to evaluate the performance of the prediction score with an independent data set collected in 2009. **Results:** We identified signs of rodent infestation in 63% (60/95) and 35% (64/184) of the cases and control households, respectively. Independent risk factors for acquiring leptospirosis in a household were rodent burrows (OR, 3.30; 95% CI, 1.50-7.26), *Rattus norvegicus* feces (2.86; 1.24-6.59), rodent runs (2.57; 1.06-6.22), household bordering an abandoned house (2.48; 1.04-6.02), and unplastered walls (2.22; 1.02-6.02). A prediction score was developed by assigning points (3, 3, 2, 2 and 2 respectively) to each risk factor. The area under the ROC curve for the scoring system was 0.70 (95% CI, 0.64-0.76) and 0.71 (0.65-0.79) for the development and validation datasets. **Conclusions:** Our study indicates that high proportions (>44%) of urban slum households are infested with *R. norvegicus*. A simple prediction score performed well when identifying high-risk households for leptospirosis within slum communities. These findings need to be confirmed in other urban centers. Yet, they suggest that community-based screening for rodent infestation can be used to target rodent and environmental control measures in populations at highest risk for leptospirosis.

INTRODUCTION

Pathogenic *Leptospira* infection produces a broad spectrum of manifestations ranging from mild and self-limited illness to severe and life-threatening disease. Case fatality rates from the most severe disease forms, Weil's disease and severe pulmonary hemorrhage syndrome, are greater than 10% and 50% respectively (1-4). Annually, more than 500,000 cases of leptospirosis occur worldwide (5). In developing countries, leptospirosis is an emerging problem in urban slum health (6). Epidemics are reported during seasonal heavy rainfall and flooding (6-14). In these poor communities, leptospirosis is associated with high flood risk, inadequate sewage systems and poor refuse collection services (4, 6, 14-16).

Human infection is mainly acquired through skin contact with water or soil contaminated by leptospiras, which are spread in the environment through the urine of mammal reservoirs (2, 17-18). Studies indentify the brown rat (*Rattus norvegicus*) carrying *Leptospira* in the vicinity of index cases and therefore rats are considered the greatest hazard for *Leptospira* transmission in urban areas (19-22). In addition, severe leptospirosis cases in larger Brazilian cities are caused predominantly by the single serovar *L. interrogans* serovar Copenhageni, which is associated with *R. norvegicus* (6, 23-25). Studies of the peridomiciliary environments in high risk areas for leptospirosis in Salvador, revealed specific factors related to rat infestation and *Leptospira* transmission, supporting the role of rodent-related household transmission. Subjective rat infestation markers such as peridomiciliary sighting of rats by residents and residence in proximity of a rat preferential environment like open sewers, were independent risk factors for severe leptospirosis (8) and presence of *Leptospira* antibodies (16).

Effective control of urban leptospirosis is hampered by the challenges of introducing large scale sanitation programs in slums early diagnosis in the absence of a point-of-care

diagnostic test (3) and the nonexistence of an effective human vaccine (26-27). Use of boots or protective clothing (28) and antibiotic prophylaxis (29-30) are difficult interventions to implement in large, chronically at-risk populations. Currently, campaigns to manage rodents which include chemical rodenticides and environmental approaches to reduce rodent food, water and harborage, are the principal strategy to prevent urban leptospirosis (31). Urban rodent management is based on surveys of households' exterior areas to obtain information on rodent infestations and infrastructural deficiencies that support rodent populations (32). However those strategies are costly and have not been standardized for use in slum areas of developing countries. Additionally, no studies have systematically examined whether variables assessed during rodent survey can be used as predictive markers for risk of leptospirosis. Targeted and cost-effective interventions specific to households with high risk for *Leptospira* transmission could improve both leptospirosis prevention and rodent management. Therefore, we examined whether readily-available characteristics of the household environment and signs of rodent infestation are associated with occurrence of severe leptospirosis in household subjects.

METHODS

Surveillance site

Salvador, capital of the Bahia state, has more than 2.9 million inhabitants and is the third most populous Brazilian city after São Paulo and Rio de Janeiro (33). Couto Maia Hospital is the reference center for infectious disease in Bahia state receiving 98% of leptospirosis cases of the metropolitan region (8). Beginning in January 2007 and for 36 months (until December, 2009) active surveillance, performed by trained personal, consecutively identified patients from Salvador city that fulfilled a clinical case definition for

severe leptospirosis (6, 8). Additionally, for laboratorial confirmation acute and convalescent-phase serum samples were collected and evaluated by microscopic agglutination test (MAT) according to previously described protocols (6). A laboratory-confirmed case of leptospirosis was defined as the presence of a four-fold rise in the MAT titer between paired acute and convalescent-phase serum samples or a titer of 1:800 in a single sample (6). Ethical clearance for this study was granted by the Ethical Committee in Research of the Oswaldo Cruz Foundation and IRB Committee of Weill Medical College of Cornell University.

Design Study

A matched case-control study was conducted in Salvador city. A household was regarded as a case household if there was at least one severe leptospirosis patient among permanent household members. For each case household that could be located two neighborhood-matched control households were selected from the same slum communities according to the sampling scheme used in two previous investigations (4, 8). Control households were sampled which were located a distance of five domiciles from the case household, and at every household thereafter, until a neighborhood control household was identified which did not have a member who was diagnosed at a health care facility as having leptospirosis in 2007/09 and agreed to participate in the study (4). This strategy was selected in order to avoid overmatching for rodent infestation characteristics between case and control households. Two control households were selected for each of the case households by sampling domiciles in opposite directions. The study team identified and recruited case and their respective control households during the same community site visit.

The case control study design was used as the base to develop and validate an environmental score for risk of leptospirosis. Cases and control households from the years

2007 and 2008 were included in the score development group. Households from 2009 were defined as the score validation group.

Data collection and definitions

Visits for cases and control households from patients identified through 2007 were performed retrospectively during December 2007 and January 2008. For patients identified in 2008 and 2009 cases and control household visits were performed prospectively with a maximum time lag after clinical leptospirosis confirmation of three weeks.

Environmental surveys of case and control households was done by the authors (N.S. and C.S.) and experienced rodent control specialists of "Centro de Controle de Zoonoses" (MoH) from Salvador during household visits. The survey team was guided by an exterior inspection form, adapted from the CDC manual (34). The form, which is available in English and Portuguese on request, was divided in eight parts: 1) 3 questions on demographic information, 2) 4 variables on premise type and details; 3) 4 variables on food source for rodents; 4) 2 variables on water source for rodents; 5) 11 variables on harborage for rodents; 6) 5 variables on entry/access for rodents; 7) 6 variables on signs of rodent infestation; and 8) 3 questions on domestic animals. Because of the environmental and socioeconomic differences found in Salvador some variables from CDC manual needed to be excluded or modified and additional variables incorporated (details are shown in Manuscript I). To measure possible environmental variations between the date of hospitalization and the environmental survey, we asked the head-of-household if domicile structure, peridomestic area, drainage systems or accumulated refuse had changed from the date of hospitalization.

Statistical analysis

Epidemiological and laboratory data were double-entered and validated using the Epi-Info for Windows software (Centers for Disease Control and Prevention, Atlanta, GA) database. A chi-square for matched data (McNemar's chi-square) and conditional regression logistic were used to compare categorical and continuous data, respectively, in the bivariable analysis to investigate: a) differences between cases households from development and validation groups and b) the association of case and control status with exposure to different environmental household characteristics in the development group. A P value of 0·05 or less was used in two sided testing as criteria for a statistically significant difference. In the development group, variables that attained a p-value<0.1 in the univariate analysis were retained for multivariable analysis using conditional logistic regression. A backward elimination strategy was performed to obtain the final model.

To develop a practical prognostic score, we assigned the independent risk factors identified by multivariate analysis, in the development group, weighted points proportional to the β regression coefficient values (rounded to the nearest integer) (35). A risk score was then calculated for each household, and the population was divided into three categories by comparing differences in sensitivity-specificity: households at low risk, households at intermediate risk, and households at high risk for leptospirosis. For both the development and the validation groups we assessed the discriminative power of the score by using c-statistics generated by receiver operating characteristic (ROC) curve, sensitivity and false positivity rate. C-statistics greater than 0.80, 0.70 to 0.79, 0.60 to 0.69, and 0.50 to 0.59 indicate excellent, good, fair, and poor predictive ability, respectively.

RESULTS

Households Characteristics

From 2007 to 2009 were registered 179 leptospirosis patients who met the clinical and laboratorial definition for leptospirosis. We were not able to survey 15 case households because they were not located (13) or had inappropriate conditions to perform the survey originated by violence activity in the neighborhood (2). We excluded two case households that did not present matched control households satisfying the selection criteria. The final number of case and control households was 162 and 315 respectively. Most of case households (153) had two matched control households and 9 cases households had only one matched control that fulfilled the selection criteria.

From the total number or cases and control households, 95 case and 184 control households were identified in 2007/08 and were assigned in the development group. Case and control households, 67 and 131 respectively, were identified in 2009 and were defined as validation group. Case households characteristics of the development and validation group are presented in Table 1. Groups presented similar characteristics and 35 of the 38 variables did not present differences. Case households in the validation group presented proportionately less abandoned appliances but had more holes in the floor and rodent burrows (Table 1).

Considering the development group, 63% of the cases and 35% of control households presented at least one rodent sign and were considered infested. Major active rodent signs were rodent burrows, runs and *R. norvegicus* fecal droppings presenting in 104 (37%), 55 (20%) and 48 (17%) of the households respectively (Table 2). Most of the rat burrows and runs apparently belonged to *R. norvegicus* because they were build on earth superficies in the proximity with sources of water. Nevertheless, *R. norvegicus* fecal droppings presented poor concordance with rodent burrows (kappa=0.31; CI = 0.22-0.40) and marginally good

concordance with rodent runs (kappa=0.41; CI = 0.29-0.53). Of the 52 households with fecal droppings, 92% presented signs of *R. norvegicus*, 6% of *M. musculus* and 0% of *R. rattus* (Table2).

Bivariable Results

We used bivariable analyses to test the ability of potential risk factors to predict the risk of leptospirosis in a household. Table 2 shows that several variables were associated with a higher risk of leptospirosis. Premise details variables like presence of open sewer<10m and household border on an abandoned house were found to be associated with case households. Food and water sources for rodent variables also were associated to case households (exposed garbage, animal food, other food and plants, open stores of human food and water leaks). Bushes or shrubbery were sources of harborage for rodents in case households. Considering variables related to the access available for rodents to enter the building, case households presented more frequently structural deficiencies, holes in floor and un-plastered walls. A larger percentage of case household (P<0.05) showed signs of rodent infestation related to *R. norvegicus*, such fecal droppings, burrows and runs. Significant associations were not found for indicators of low socioeconomic status as per capita income, number of inhabitants in the house and proportion of males. Additionally, the risk of acquiring leptospirosis in a household was associated with domestic animals, specifically presence of chickens.

Multivariate Results and Development of Prediction Models

To identify independent predictor variables for the development group, we performed multivariate analysis (Table 3). The final model retained five variables: three variables from the group of rodent infestation factors, one from the group of premise details variables and other one from access to rodents group. Rodent burrows had the strongest association with

case households in the model (OR = 3.30, 95% CI = 1.50-7.26). *R. norvegicus* fecal droppings (OR = 2.86, 95% IC = 1.24-7.26) and rodent runs (OR = 2.57, 95% IC = 1.06-6.22) were additional independent risk factors related to rodent infestation. Household bordering on an abandoned house and un-plastered exterior wall surface presented OR = 2.48 (95% IC = 1.02-6.02) and OR = 2.22 (95% IC = 1.02-6.02) respectively. An additional model was created replacing the variables fecal droppings, rodent burrows and runs for the combined variable of any sign of rat infestation. This model retained only the variable of any sign of rat infestation (OR = 4.91, 95% CI = 2.69-9.75).

To calculate a risk score based on the development group, we assigned each of the six prognostic variables a number of points that was proportional to its regression coefficient (Table 3). A score was calculated for each household by adding together the points corresponding to its risk factors. The households were then divided into 11 subgroups on the basis of the score which ranged from 0 to 12 (there were not households in the point subgroups 1 and 11) (Table 4). 32% of the case households and 63% of the control households had a point value of 0. Because score values were not normally distributed within case and control households, we used Wilcoxon ran-sun test to compare the scores by case status. The median risk score for case households was statistically significantly different from that for control households (5 and 2, respectively; $p<0.001$). Score cumulative sensitivity and false positivity rate for the 11 subgroups were used to define 3 groups with significantly different risk: a low-risk group (0 to 2 points), an intermediate-risk group (3 to 5 points), and a high-risk group (6 to 12 points) (Table 4). The development group yielded a *c* statistic of 0.70 (95 percent confidence interval: 0.63-0.76),

Model Validation

The score was calculated for the validation group where 12% of the case households and 40% of the control households had a point value of 0. As the validation group, the median risk score for case households was also significantly different from that for control households (3 and 0, respectively; $p<0.001$). The validation group yielded a *c* statistic of 0.71 (95 percent confidence interval: 0.65-0.79). There were not differences in the two scores' ability to discriminate between case and control households (p=0.38). ROC curves were plotted in the Figure 1.

DISCUSSION

Efforts to implement and improve rodent management interventions for urban leptospirosis have been hampered by the lack of readily available information and epidemiologically-based markers that allow identification and monitoring of households at increased risk for leptospirosis. Our study demonstrates that the risk of acquiring leptospirosis in a given household in a slum urban areas is predicted by the presence of five variables related to objectives signs of rodent infestation and environmental features. In descending order of importance, these features are rodent burrows, *R. norvegicus* fecal droppings, rodent runs, borders on an abandoned house and un-plastered walls. A risk score derived by combining points for each of these features accurately classified households into subgroups at low, medium, and high risk for occurrence of leptospirosis. Households in the medium or high risk groups could potentially benefit the most from aggressive chemical rodent control, environmental interventions and educational measures.

Specific markers of rodent infestation were strongly associated with the occurrence of leptospirosis in a household. Those results support the findings described in Manuscript I where the same markers were related with *Leptospira* infection. Sixty percent of the case houses had signs of rodents, whereas only 35% of control houses had those signs. Additionally, our results support previous findings that leptospirosis is transmitted in the household environment of slum population residing in developing countries (4, 8, 16).

The rodent infestation rate between surveyed households was 44%. Although the present study was not specifically developed to evaluate rodent infestation, our findings suggest significant infestation in those neighborhoods. It also reflects the general trend seen in urban areas around the world (36-38), where the poorer the conditions, the higher the level of infestation (39).

In line with previous work, which identified *Rattus norvegicus* as the dominant species in a neighborhood of Salvador (Manuscript I), *R. norvegicus* was the predominant rodent species in the study area. This pattern is also observed in other urban areas (36-37, 40-45). In agreement with findings of a previous study *R. norvegicus* fecal droppings and rat burrows were independent risk factor for *Leptospira* transmission in a household. Evidence of signs of *R. norvegicus* as risk factor for leptospirosis in addition to the high infestation rate of this species reinforce the key role of the brown rat for leptospirosis transmission in urban areas. Additionally, a high level of *Leptospira* serovar Copenhagen carriage in *R. norvegicus* has been shown (22). The identification of a unique predominant rodent reservoir in urban habitats at risk of leptospirosis transmission, highlights the importance of implementing targeted management interventions based in *R. norvegicus* ecology.

The premise details variable describing border on an abandoned house was an independent risk factor. Abandoned houses provide harborage for rodents (46). Also, abandoned houses can act as a socioeconomic factor indicating environmental deterioration because in areas highly populated where houses are rapidly occupied only structures almost destroyed are abandoned. Open sewer proximity, which was not retained in the model, could be a risk factor for *Leptospira* infection or could be the rodents' source of water in an infestation. It has been shown that *R. norvegicus* prefers environments with free water and has been associated to sewers (38, 47-48). The strong relation between rodent infestation and risk of *Leptospira* infection in a household probably covers the role of open sewer as risk factor for leptospirosis transmission.

We observed a large availability of concomitant food, harborage and access sources for rodents in the neighborhoods studied. In these complex and saturated habitats, variables which favor household rodent infestation were not appropriate to predict leptospirosis high risk households. The only independent risk factor belonging to these groups was un-plastered

wall, a variable initially proposed for rodent access. This variable was also associated with *Leptospira* infection in a previous study (Manuscript I) and we believe un-plastered wall may be a proxy for socioeconomic status not captured by the income variable.

This is the first environmental score that can be used to stratify risk groups for leptospirosis in households of urban setting. The score, based on five risk factors and an additive point system, performed well in stratifying households into risk groups for severe leptospirosis. Additionally, the score was successfully validated in an independent sample with no decrease in discrimination. Our score is easy to use while maintaining a moderate prognostic accuracy of 0.7. Score sensitivity and specificity at a point value of 3 (intermediate risk) were 68% and 63% respectively. Environmental survey tools to identify the highest sensitivity combined with an operating point that provides the lower proportion of false negative results. Specificity of 50% was defined, in *Aedes aegypti* programs, as the lower limit to decrease the number of units falsely classified at risk, which activates unnecessary interventions and generates unproductive costs (49).

Large cities in Brazil, have recently implemented rodent control programs aimed to decrease leptospirosis incidence. Interventions are variable but most of the programs focus in prioritizing defined areas with high disease incidence. Those areas are large (20,000 to 60,000 households) and include a high socioeconomic and environmental heterogeneity between households. Rodent control programs are cost-time expensive because they require mass rodent infestation screening and treatment of the households inside the areas three times a year. Because the low visibility of leptospirosis and the recent implementation of rodent control programs, their continuity and size are frequently negatively affected during epidemics of other diseases (mainly dengue) and the lack of resources. Using mass rodent infestation screening that is already performed, interventions prioritizing rodent chemical control in households with score point values ≥3 could be more cost effective than

conventional interventions. Additionally, households classified by degree of risk for leptospirosis could help policymakers to implement more focused rapidly interventions during outbreaks or during periods with lack of resources.

Our model has several limitations. First, the study was limited by the time lag between occurrence of leptospirosis and assessment of housing rodent survey in households during 2007. Exclusion of households with modifications in domicile structure, peridomicile, open sewer or refuse deposit can control this bias, but other unregistered environmental factors could be changed during the study period in the households included during the year 2007. Second, we tried to control observational bias through a predefined structured questionnaire, additionally surveyors did not know the aims of the study. Third, the distance of 50 m between cases and control households to avoid overmatching related to rodent infestation variables may have inadvertently masked other risk factors. Fourth, the results of the present study may not be generalizable to other urban settings; however 37% of the Brazilian urban population resides in slums with equal or greater levels of poverty as found in the study neighborhoods (50). Additionally, a large proportion of the world slums population resides in conditions of poverty and environmental degradation that support high levels of rat infestations similar that in Salvador.

In conclusion, we developed and validated a risk score based on five variables related to objectives signs of rodent infestation and environmental features that predicts risk of leptospirosis occurrence in a household among houses of slum urban areas. These findings may be useful in developing rodent management programs to predict individual household probabilities, to direct control measures and for policymakers to allocate limited health care resources.

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CONFLICT OF INTEREST

We declare that none of the authors have any potential conflict of interest.

FIRST AUTHOR"S BIOGRAPHICAL SKETCH

MSc Federico Costa is a biologist and PhD student at the Oswaldo Cruz Foundation, Brazilian Ministry of Health in Salvador, Brazil, whose research interests focus on infectious disease problems and reservoirs ecology that affect urban slum populations.

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Table 1: Rodent-related and environmental characteristics among 95 and 67 case households of development and validation groups in Salvador, Brazil.

Household characteristics	**Development*** (***n*** **= 95)**	**Validation*** (***n*** **= 67)**	
	No. (%) or median (IQR)†		***P*‡**
Demographics			
No. of inhabitants	4 (3-5)	3 (2-5)	-
Male sex	2 (1-2)	1 (1-2)	-
Per capita income, US$/d	2.6 (1.3-4.1)	3.1 (2.1-5.2)	-
Premise type and details§			
Residential use only§	92 (97)	63 (94)	-
Borders on a vacant lot	18 (19)	5 (6)	-
Open sewer <10m distance	30 (32)	24 (36)	-
Borders on an abandoned house	22 (23)	13 (20)	-
Access to food sources§			
Exposed garbage§	78 (82)	60 (89)	-
Animal food§	45 (47)	31 (46)	-
Other food & plants§	64 (67)	39 (58)	-
Open stores of human food	52 (55)	27 (40)	-
Access to water§			
Standing water§	24 (25)	20 (30)	-
Leaks§	34 (36)	27 (40)	-
Harborage for rodents§			
Abandoned vehicles§	1 (0)	0 (0)	-
Abandoned appliances§	94 (99)	56 (87)	<0.01

Lumber/clutter on ground§	67 (70)	44 (65)	-
Other large rubbish§	44 (46)	35 (52)	-
Outbuildings/Privies§	19 (20)	20 (30)	-
Dilapidated fences & walls§	19 (20)	18 (27)	-
Plant-related§	75 (79)	56 (83)	-
Bushes or shrubbery	42 (44)	28 (42)	-
Ornamental plants	65 (68)	54 (81)	-
Presence of exposed earth	61 (64)	45 (67)	-
Built on earthen slope¶	50 (53)	34 (51)	-
Entry/Access§			
Structural deficiencies§	64 (67)	44 (65)	-
Hole(s) in roof	50 (52)	30 (45)	-
Hole(s) in wall	29 (30)	21 (31)	-
Hole(s) in floor	19 (20)	26 (39)	<0.05
Un-plastered walls#	64 (67)	41 (61)	-
Rodent active signs§			
Active signs§	60 (63)	35 (52)	-
Rodent burrows	53 (56)	22 (33)	<0.01
Rodent runs	33 (35)	20 (30)	-
R. norvegicus feces	28 (29)	18 (27)	-
R. rattus feces	0 (0)	1 (1)	-
M. musculus feces	1 (1)	2 (2)	-
Domestic animals			
Dogs	39 (45)	27 (40)	-
Cats	13 (13)	6 (9)	-

Chickens	13 (13)	9 (13)	-

* Case households in which laboratory-confirmed cases of leptospirosis resided during the periods January 2007 – December 2008 (development cohort) and January 2009 – December 2009 (validation cohort).

† Median and inter-quartile range (IQR) values are shown for continuous variables.

‡ Values are not shown for non-significant associations in matched analyses.

§ Categories and variable defined in the CDC form (34).

¶ Presence of exposed earth slope (>45º) within 10m of the household.

Walls composed of exposed bricks without external application of stucco or plastering.

Table 2: Rodent-related and environmental risk factors for severe leptospirosis among 95 case and 184 control households in Salvador, Brazil.

Household characteristics	**Case*** **(*n* = 95)**	**Control*** **(*n* = 184)**	
	No. (%) or median (IQR)†		***P*‡**
Demographics			
No. of inhabitants	4 (3-5)	4 (2-5)	-
Male sex	33 (33-50)	36 (25-50)	-
Per capita income, US$/d	2.6 (1.3-4.1)	2.8 (1.6-4.6)	-
Premise type and details§			
Residential use only§	92 (97)	176 (96)	-
Borders on a vacant lot	18 (19)	37 (20)	-
Open sewer <10m distance	30 (32)	31 (17)	<0.05
Borders on an abandoned house	22 (23)	24 (13)	<0.05
Access to food sources§			
Exposed garbage§	78 (82)	174 (73)	<0.05
Animal food§	45 (47)	66 (36)	<0.05
Other food & plants§	64 (67)	101 (55)	<0.05
Open stores of human food	52 (55)	74 (40)	<0.01
Access to water§			
Standing water§	24 (25)	45 (24)	-
Leaks§	34 (36)	41 (22)	<0.01
Harborage for rodents§			
Abandoned vehicles§	1 (0)	0 (0)	-
Abandoned appliances§	94 (99)	183 (99)	-

Lumber/clutter on ground§	67 (70)	112 (61)	-
Other large rubbish§	44 (46)	94 (51)	-
Outbuildings/Privies§	19 (20)	31 (17)	-
Dilapidated fences & walls§	19 (20)	32 (17)	-
Plant-related§	75 (79)	147 (80)	-
Bushes or shrubbery	42 (44)	61 (33)	<0.05
Ornamental plants	65 (68)	168 (70)	-
Presence of exposed earth	61 (64)	102 (55)	-
Built on earthen slope¶	50 (53)	88 (48)	-
Entry/Access§			
Structural deficiencies§	64 (67)	96 (52)	<0.05
Hole(s) in roof	50 (52)	75 (41)	-
Hole(s) in wall	29 (30)	38 (21)	-
Hole(s) in floor	19 (20)	18 (10)	<0.05
Un-plastered walls#	64 (67)	99 (53)	<0.05
Rodent active signs§			
Active signs§	60 (63)	64 (35)	<0.001
Rodent burrows	53 (56)	51 (27)	<0.001
Rodent runs	33 (35)	22 (12)	<0.001
R. norvegicus feces	28 (29)	20 (11)	<0.001
R. rattus feces	0 (0)	0 (0)	-
M. musculus feces	1 (1)	3 (2)	-
Domestic animals			
Dogs	39 (45)	62 (33)	-
Cats	13 (13)	18 (10)	-

Chickens	13 (13)	12 (6)	<0.05

* Case and control households comprised of respectively, households in which laboratory-confirmed cases of leptospirosis resided and neighborhood households which were located within 35 to 50m of case households and did not have a member who developed leptospirosis during the study period.

† Median and inter-quartile range (IQR) values are shown for continuous variables.

‡ Values are not shown for non-significant associations in matched analyses.

§ Categories and variable defined in the CDC form (34).

¶ Presence of exposed earth slope (>45º) within 10m of the household.

Walls composed of exposed bricks without external application of stucco or plastering.

Table 3: Logistic regression analysis of rodent-related and environmental risk factors for severe leptospirosis and scoring system.

Variables	**Matched OR (95% CI)***		***β* regression coefficient**	**Points†**
	Unadjusted	**Adjusted**		
Rodent burrows	5.81 (2.79-12.11)	3.30 (1.50-7.26)	1.19	3
Rodent runs	5.83 (2.61-13.00)	2.57 (1.06-6.22)	0.94	2
R. norvegicus feces	3.76 (1.84-7.64)	2.86 (1.24-6.59)	1.05	3
Borders abandoned house	2.50 (1.16-5.36)	2.48 (1.02-6.02)	0.90	2
Unplastered walls‡	2.11 (1.12-3.98)	2.22 (1.02-6.02)	0.79	2

* Mantel-Haentzel odds ratios (OR) and 95% confidence intervals (CI) are shown for matched analyses. Conditional logistic regression was performed to obtain estimates for odds ratios which were adjusted for covariates in the final model.

† Assignment of points to risk factors was based on a linear transformation of the corresponding β regression coefficient. The coefficient of each variable was divided by 0.79 (the lowest β value, corresponding to un-plastered walls), multiplied by two, and rounded to the nearest integer.

‡ Walls composed of exposed bricks without external application of stucco or plastering.

Table 4: Score system sensitivity and false positivity rate in the development cohort.

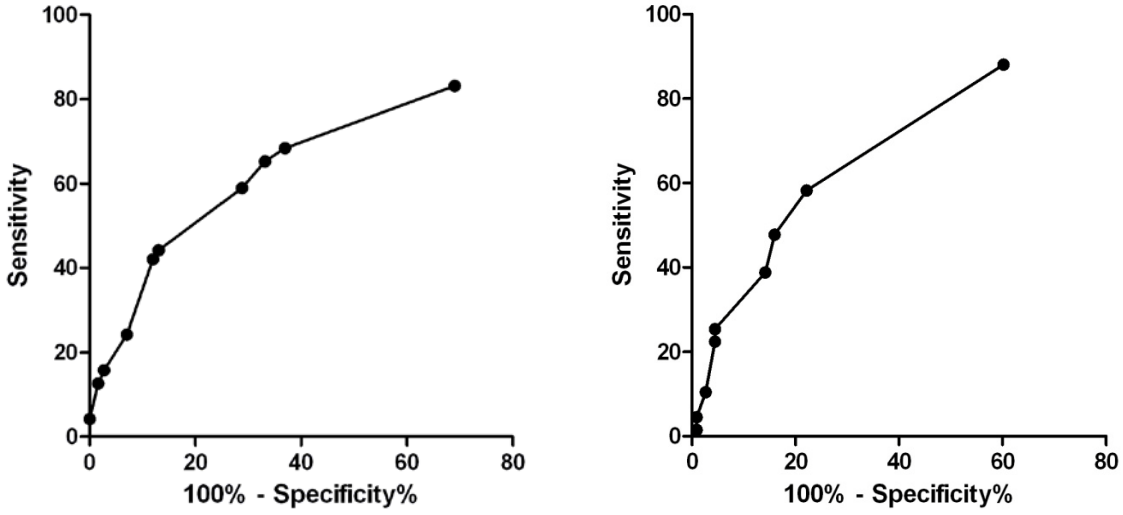
Risk categories (Score points)	**Development Group**				**Validation Group**			
	Case household (% total)	**Control household (% total)**	**Cumulative sensitivity (95% CI)**	**Cumulative false positive rate (95% CI)**	**Case household (% total)**	**Control household (% total)**	**Cumulative sensitivity (95% CI)**	**Cumulative false positive rate (95% CI)**
Low (0-2 points)	30 (32)	116 (63)	100	100	28 (42)	88 (78)	100	100
Intermediate (3-5 points)	24 (24)	43 (24)	68 (58-77)	37 (30-45)	22 (33)	20 (18)	58 (45-70)	22 (15-31)
High (6-12 points)	42 (44)	24 (13)	44 (34-54)	14 (9-19)	17 (25)	5 (4)	25 (15-37)	4 (2-10)

Figure Legends

Figure 1. Receiver operating characteristic curve for based logistic regression model score system. Area under the curves for the developing cohort 0.70 (95% CI 0.63-0.76) and for the validation cohort 0.71 (95% CI 0.65-0.79)

Figure 1

A **B**



5. DISCUSSÃO

Poucos estudos têm intentado definir fatores ambientais do domicílio associados com a transmissão peridomiciliar da *Leptospira* em comunidades carentes de Brasil (MACIEL et al., 2008; REIS et al., 2008; SARKAR et al., 2002). Nenhum destes estudos integrou, em seus desenhos epidemiológicos, a inspeção ambiental sistemática da infestação por roedores e a confirmação laboratorial prospectiva do patógeno. Neste trabalho apresentaram-se evidências provando que, após ajustar os fatores de interferência, marcadores específicos da infestação de roedores estão fortemente associados à transmissão da leptospirose. Mais ainda, os mesmos marcadores repetem-se para os dois tipos de desfecho estudados neste trabalho, a infecção por *Leptospira* e as apresentações graves da leptospirose.

Variáveis objetivas relacionadas à infestação de roedores não tinham sido analisadas em estudos epidemiológicos associados à leptospirose. "Fezes de *R. norvegicus*" e "tocas" foram fatores de risco independentes que apresentaram maior associação com infecção por *Leptospira* e leptospirose grave. A variável "trilhas de roedores" foi fator independente para a leptospirose grave. Assim como para outras doenças (BONNER et al., 2007; CHILDS et al., 1995), a ausência de sinais de roedores no peridomicilio é um bom indicador de baixa probabilidade de transmissão da leptospirose. Adicionalmente, estes resultados sustentam dados prévios que descrevem a leptospirose como uma doença de transmissão domiciliar em comunidades carentes de países em desenvolvimento (MACIEL et al., 2008; REIS et al., 2008; SARKAR et al., 2002).

Foram identificados elevados índices de infestação por roedores no bairro do Pau da Lima (46%) e em outros bairros de Salvador (44%). As taxas de infestação registradas condizem com resultados achados pelo CCZ que durante uma avaliação de 70.000 imóveis de 9 áreas de risco para leptospirose, no ano 2009, registrou uma taxa geral de infestação de 38% (dados não publicados). Estes elevados índices de infestação nos sítios de estudo não são surpreendentes já que não existiam medidas sistemáticas de controle de roedores durante o período trabalhado. A proporção de domicílios infestados nos bairros de Salvador foi similar às descritas por outros estudos em áreas carentes de América latina (CAMERO, 2004; DE MASI ;VILACA ;RAZZOLINI, 2009; FERNÁNDEZ, 2007; VILLAFAÑA, 2000), mas, superior às achadas em países desenvolvidos (CHILDS, 1998; DEFRA, 2005; EASTERBROOK et al., 2005).

Com base no registro de sinais de infestação, *R. norvegicus* foi o roedor dominante nas áreas de estudo em Salvador. A predominância de *R. norvegicus* tem sido previamente descrita em outras áreas urbanas de cidades da América Latina (CAVIA, 2009; FERNÁNDEZ, 2007; VILLAFAÑA, 2000), América do Norte (CHILDS et al., 1995; EASTERBROOK et al., 2005) e Europa (BAJOMI, 1986; IERADI, 1996; TRAWEGER, 2005). Entretanto nas cidades de Tumero, Venezuela (CAMERO, 2004) e São Paulo (DE MASI ;VILACA ;RAZZOLINI, 2009), o *R. rattus* foi a espécie dominante. O fato de que sinais indicadores da presença de ratos de esgoto num domicílio sejam fatores de risco para a transmissão da leptospirose reforça o papel do *R. norvegicus* como o principal reservatório desta doença em ambientes urbanos. Estes resultados estão alinhados com três trabalhos que realizaram captura de roedores em ambientes urbanos de Salvador com transmissão de leptospirose. Em Salvador na década de 1950 (ANDRADE ;OLIVEIRA, 1955), em 1998 (DE FARIA et al., 2008) e 2010 (dados não publicados) foram registradas taxas de 29%, 82% e 61% de prevalência de portador de *Leptospira* entre *R. norvegicus* capturados. A identificação de um reservatório predominante em ambientes urbanos de elevado risco de transmissão da leptospirose remarca a importância da implementação de intervenções focadas na ecologia de *R. norvegicus*.

Além das variáveis próprias da infestação de roedores avaliou-se também uma série de variáveis ambientais relacionadas à presença de roedores como “refúgio”, “alimento e água” e “acesso ao domicílio”. A variável “água disponível” esteve independentemente associada à infecção por *Leptospira*. A mencionada variável, que também inclui água de esgoto, poderia ser um fator de risco para a transmissão da doença uma vez que outros estudos epidemiológicos amostraram relação entre esgotos e transmissão da leptospirose (REIS et al., 2008; SARKAR et al., 2002). Adicionalmente, a presença de água pode estar relacionada diretamente com a infestação por roedores devido a que o *R. norvegicus* prefere ambientes com elevada disponibilidade de água, especialmente esgotos (CHANNON ;COLE ;COLE, 2000; DE MASI ;VILACA ;RAZZOLINI, 2009; LANGTON, 2001). A segunda variável ambiental relacionada à transmissão da doença foi “casa abandonada”, fator de risco independente para leptospirose grave. Casas abandonadas representam fonte de refúgio para roedores (DEFRA, 2005). Também, casas abandonadas podem atuar como um marcador socioeconômico indicando deterioração ambiental, já que em áreas densamente povoadas, onde domicílios são invadidos rapidamente, somente locais quase destruídos são abandonados. A terceira variável ambiental “muro não rebocado” esteve independentemente associada tanto à infecção por *Leptospira* como à leptospirose grave. Esta variável foi

inicialmente proposta como variável de acesso do roedor ao domicilio já que facilita a ação de trepar de algumas espécies como o *R. rattus*. Devido ao fato de o roedor predominante achado, *R. norvegicus*, preferir ambientes peridomiciliares e não ser definido como bom trepador acredita-se que esta variável pode ter atuado como marcador de status socioeconômico não capturado pela variável "renda per capita"ou como indicador de comportamento de cuidado da casa.

Com relação às variáveis socioeconômicas, só a variável "renda per capita" foi fator de risco independente para infecção por *Leptospira*, mas não esteve relacionada à leptospirose grave. Uma renda baixa é considerada no Brasil como um indicador de desigualdade da saúde (BARROS ;VICTORA ;HORTA, 2001) e tem sido associada ao risco de transmissão da leptospirose no passado (REIS et al., 2008). Baixa renda pode estar relacionada a fatores de risco, como limpar esgotos depois de alagamentos produzidos pelas chuvas, uso limitado de roupas de proteção que podem reduzir o risco de entrada da espiroqueta *Leptospira*, ou piores condições de higiene no domicílio favorecendo as condições causativas da presença de roedores.

O escore desenvolvido no manuscrito 2 é a primeira ferramenta destas características que pode ser utilizada para estratificar grupos de risco para leptospirose em domicílios de ambiente urbano. O escore, baseado em cinco características ambientais e um sistema de pontos aditivos, é fácil de usar e manteve uma acurácia prognóstica moderada, com valor de área sob a curva ROC de 0.7. Adicionalmente, o escore foi exitosamente validado numa amostra independente mantendo seu poder discriminatório. A sensibilidade e a especificidade do escore no valor de 3 (risco intermediário) foram de 68% e 63% respectivamente. As ferramentas de vigilância ambiental estão orientadas a identificar a maior sensibilidade combinada com um ponto operativo que provia a menor proporção de resultados falso-negativos. Uma especificidade de 50% foi definida nos programas de controle do *Aedes aegypti*, como o valor limite para diminuir o número de unidades falsamente classificadas em risco, as quais geram intervenções desnecessárias e geram custos improdutivos (SANCHEZ et al., 2006).

Cidades do Brasil, como São Paulo, Salvador, Recife e Curitiba têm realizado nos últimos cinco anos programas de controle de roedores com o objetivo de diminuir a incidência da leptospirose. A metodologia dos programas varia de cidade para cidade, mas a maioria dos programas prioriza áreas de alto risco de transmissão da doença. Estas áreas de intervenção dependem da capacidade operativa dos CCZs locais, que varia entre 20.000 e 60.000 domicílios. Dentro destas áreas existe uma elevada heterogeneidade ambiental e

socioeconômica entre os domicílios. Os programas de controle de roedores são custosos em recursos e tempo já que requerem três ciclos anuais, sendo que para cada ciclo devem ser realizadas as seguintes ações: 1) inspeção ambiental de todos os domicílios e imediata aplicação do primeiro pulso de rodenticida, 2) um segundo pulso aplicado 15 dias após o primeiro, 3) um terceiro pulso aplicado 30 dias após o primeiro, e 4) inspeção ambiental de uma amostra de domicílios dois meses após o primeiro pulso. Devido ao fato de a leptospirose ser uma doença negligenciada, a recente implementação dos programas de controle de roedores, frequentemente são interrompidos durante surtos de outras doenças (principalmente dengue) ou ainda por falta de recursos. Tomando como base os programas de vigilância ambiental que já foram implementados, a aplicação de intervenções que priorizem o controle químico em domicílios com escore ≥3 teriam melhores resultados no custo-efetivo quando comparados com as estratégias atualmente utilizadas. Adicionalmente, os domicílios classificados numa escala de risco para leptospirose poderiam ajudar aos gestores a realizarem intervenções rápidas e focalizadas durante surtos ou períodos com falta de recursos.

Os resultados apresentados estão sujeitos a limitações próprias dos desenhos de estudo utilizados. Limitações especificas do manuscrito 1 foram: a) tempo de demora entre o desfecho (infecção por *Leptospira*) e avaliação ambiental. Domicílios apresentando quatro tipos de modificações ambientais (modificações na estrutura do domicílio, no peridomicílio, nos esgotos ou no acúmulo de lixo) durante o período de demora não apresentaram diferenças significativas com domicílios sem estas modificações, porém outros fatores ambientais não avaliados poderiam ter mudado durante este intervalo de tempo; b) devido à definição estabelecida para domicílio controle somente 186 (32%) de 569 controles potenciais estiveram disponíveis para randomização. Este número reduzido de controles pode ter afetado a randomização durante a seleção dos domicílios controles. Adicionalmente, uma distância de 50m entre domicílios-casos e controles para evitar a superposição de fatores de risco relacionados a roedores pode ter escondido outros fatores de risco. As limitações específicas do manuscrito 2 foram: a) no ano 2007 existiu uma demora de até um ano entre a ocorrência do desfecho (leptospirose grave) e a avaliação ambiental. Para controlar esse viés compararam-se domicílios com e sem modificações ambientais durante esse período sendo que, não foram registradas diferenças significativas entre grupos, b) os entrevistadores conheciam o status do caso ou o controle dos domicílios. Tentou-se controlar este possível viés observacional por meio da utilização de questionários padronizados. Os resultados apresentados nos manuscritos 1 e 2 podem não ser generalizáveis a outras áreas urbanas,

entretanto 37% da população urbana do Brasil vive em condições de pobreza similares às dos bairros estudados (UN-HABITAT, 2003).

Na prática, futuros estudos são imprescindíveis para validar estes achados em outras áreas urbanas onde a leptospirose seja endêmica. Além disso, a identificação de adicionais marcadores ambientais específicos auxiliaria no desempenho deste primeiro modelo com a finalidade de obter uma maior relação sensibilidade-especificidade. Os dois manuscritos apresentados confirmam achados de estudos anteriores realizados em relação à doença grave (KO et al., 1999; MACIEL et al., 2008; SARKAR et al., 2002) presença de anticorpos (REIS et al., 2008) e abrem uma oportunidade de implementação de intervenções mais eficientes contra a transmissão da leptospirose.

6. CONCLUSÕES

- Os resultados apresentados nesta tese evidenciam uma elevada infestação por *R. norvegicus* nas comunidades carentes estudadas. Esta infestação é similar a achada em outros países em desenvolvimento, porém maior que a registrada em países desenvolvidos.

- Marcadores específicos desta infestação como a presença de fezes de *R. norvegicus*, tocas e trilhas foram os principais fatores de risco preditivos para a infecção assintomática por *Leptospira* assim como também para a doença grave por leptospirose. Estes marcadores ambientais específicos são de fácil identificação nos peridomicílios de áreas com elevado risco de transmissão da leptospirose.

- Utilizando sinais da infestação por roedores foi desenvolvido e validado um escore preditivo que identifica domicílios de elevado risco dentro de comunidades com transmissão endêmica de leptospirose.

- Estes achados sugerem que a triagem da infestação por roedores e a identificação de domicílios de risco, podem ser estratégias para dirigir intervenções de controle de roedores em populações de risco para leptospirose.

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8. ANEXOS

Manuscritos e documentos publicados durante o doutorado relacionados a outros projetos

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Clinical characteristics and risk factors of human leptospirosis in Argentina (1999–2005)

N.B. Vanasco[a,b,*], M.F. Schmeling[a], J. Lottersberger[b], F. Costa[c], A.I. Ko[c,d], H.D. Tarabla[e]

[a] Instituto Nacional de Enfermedades Respiratorias (INER "Dr. E. Coni"), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS "Dr. Carlos G. Malbrán"), Santa Fe, Argentina
[b] Facultad de Bioquímica y Ciencias Biológicas, Santa Fe, Argentina
[c] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
[d] Division of International Medicine and Infectious Disease, Weill Medical College of Cornell University, NY, USA
[e] INTA, Rafaela, Argentina

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ABSTRACT

There is scarce data on the burden of leptospirosis and its epidemiological characteristics in Argentina. This study aimed to evaluate distribution of leptospirosis cases and identify risk factors for the disease during national laboratory-based surveillance. From January 1999 to December 2005, 812 suspected cases were referred to the national reference laboratory, of which 182 and 463 had respectively, laboratory confirmed and unconfirmed diagnosis of leptospirosis. The diagnosis of leptospirosis was discarded in 167 cases. The most prevalent presumptive infecting serogroup was Icterohaemorrhagie followed by Pomona, Ballum and Canicola. The majority of cases occurred during the worm and rainy months. Confirmed cases were predominantly adults and males, who presented with fever, headache and myalgias. Severe clinical manifestations included jaundice and acute renal insufficiency. Conjunctival suffusion, a hallmark clinical sign of leptospirosis, was found in 55% of confirmed cases, and 43% of the cases with discarded diagnosis ($p = 0.036$). After multivariate analyses, age >30 years (OR = 2.16; 1.05–4.41), occupation in a rural setting (OR = 3.41; 1.45–8.06), contact with contaminated surface water (OR = 2.17; 1.01–4.68), and contact with floods (OR = 4.49; 1.17–17.25) were significantly associated with leptospirosis. In conclusion, although activities associated with rural occupations remain important risk factors in Argentina, exposures occurring during flooding events have emerged to be the major risk factor for leptospirosis.

1. Introduction

Leptospirosis caused by spirochetes of the genus *Leptospira*, has a worldwide distribution and is acquired through either direct or indirect contact with urine of infected animals (Bharti et al., 2003; WHO, 2003; McBride et al., 2005). There is a wide spectrum of human disease associated with leptospirosis, ranging from subclinical infections to severe multi-organ failure associated with high mortality (Farr, 1995; Bharti et al., 2003; McBride et al., 2005). The incidence of disease is highest in humid and warm climates (Farr, 1995; Levett, 2001). The occurrence of seasonal outbreaks of leptospirosis following floods is frequent in Central and South America (Levett, 2001; Vanasco et al., 2000b, 2002, 2004; AAVLD, 2002).

In Argentina, leptospirosis was first recognized and reported in humans in 1915 in the Province of Santa Fe (Cacchione et al., 1975). However, there is limited data on the current disease burden of leptospirosis in Argentina. Furthermore the factors influencing transmission of leptospirosis in this country is not well understood (AAVLD, 2002). The reported incidence of leptospirosis is highest in regions which have access to laboratories which perform serologic confirmation with the microscopic agglutination test. In fact, the highest incidence rates for leptospirosis in Argentina are found in provinces of Buenos Aires and Santa Fe, which have established reference centres for diagnosis (AAVLD, 2002). To date, there has been little information for national laboratory-based surveillance. In this study, we describe the findings of surveillance for leptospirosis in Argentina, which was conducted by national reference laboratory in Santa Fe from 1999 to 2005. In addition to examining the burden of leptospirosis in Argentina, we evaluated the clinical presentation of cases and risk factors for acquisition of the disease.

* Corresponding author at: Instituto Nacional de Enfermedades Respiratorias (INER "Dr. E. Coni"), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS "Dr. Carlos G. Malbrán"), Blas Parera 8260, Santa Fe 3000, Argentina. Tel.: +54 342 4892830; fax: +54 342 4892830.
E-mail addresses: bibi_vanasco@hotmail.com, jlotters@fbcb.unl.edu.ar (N.B. Vanasco).

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ECOHEALTH

Original Contribution

Temporal and Spatial Host Abundance and Prevalence of Andes Hantavirus in Southern Argentina

Francisco J. Polop,[1,2] María C. Provensal,[2] Noemí Pini,[3] Silvana C. Levis,[3] José W. Priotto,[1,2] Delia Enría,[3] Gladys E. Calderón,[3] Federico Costa,[4] and Jaime J. Polop[2]

[1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[2]*Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, Agencia Postal No. 3, 5800 Río Cuarto, Córdoba, Argentina*
[3]*Instituto Nacional de Enfermedades Virales Humanas (INEVH), Pergamino, Argentina*
[4]*Fundación Mundo Sano, Buenos Aires, Argentina*

Abstract: Andes virus (AND) is a hantavirus hosted by the sigmodontine rodent *Oligoryzomys longicaudatus* in southern Argentina, where it is responsible for most cases of hantavirus pulmonary syndrome (HPS). Our study provides data about the spatial variation in abundance of the rodent host of AND hantavirus. We report results of a longitudinal study performed in a locality of the Andean region of Chubut Province. From November 2003 (spring) to July 2006 (winter), *O. longicaudatus* was the most common species captured (63%) and it showed significant differences in abundance among habitats and seasons. Most antibody-positive rodents were *O. longicaudatus* (9.2%), followed by *A. longipilis* (3.6%) and *A. olivaceus* (1.5%). The highest number of antibody-positive animals was observed for males that belonged to the heaviest mass classes. Antibody-positive *O. longicaudatus* were more abundant in brush habitats. We found low richness of rodents and abundance of *O. longicaudatus* in areas affected by anthropogenic activity. The infection seems to be regionally persistent, but the risk to humans in a landscape would be localized. To develop accurate models for predicting HPS outbreaks, further research is needed to characterize rodent movement patterns across the landscape.

Keywords: *Oligoryzomys longicaudatus*, abundance fluctuations, hantavirus pulmonary syndrome

INTRODUCTION

Hantavirus pulmonary syndrome (HPS) is a human disease caused by some members of the genus Hantavirus, family Bunyaviridae. Some hantaviruses are known to be highly pathogenic for humans (e.g., Sin Nombre virus (SNV), Bayou virus, Black Creed Canal virus, New York virus (Nichol et al., 1993; Childs et al., 1994; Khan et al., 1995; Morzunov et al., 1995; Rollin et al., 1995). Each distinct form of the virus is closely associated with a single, or possibly a few, rodent species belonging to Murinae, Arvicolinae, and Sigmodontinae subfamilies within the Rodentia Order (Schmaljhon and Hjelle, 1997; Enría and Levis, 2004). The virus is maintained in nature by transmission among rodent populations, which may occur through fighting and social grooming (Young et al., 1998), and it is transmitted to humans through aerosolized particles from contaminated excreta of rodents (Glass, 1997; Mills, 1999; Douglas et al., 2001; Padula et al., 2004).

Correspondence to: María C. Provensal, e-mail: cprovensal@exa.unrc.edu.ar

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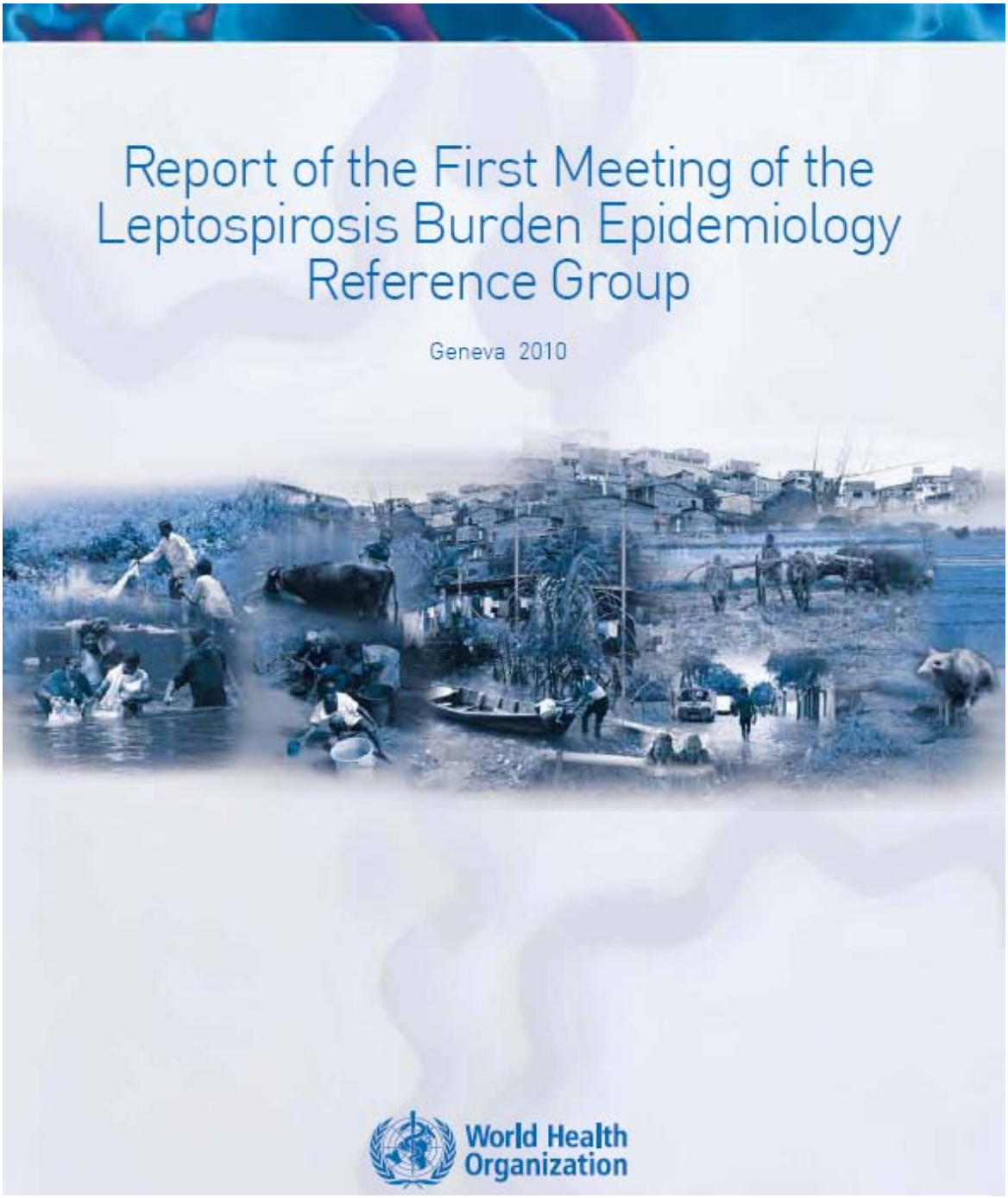

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Questionários

Questionário ambiental para a avaliação da infestação de roedores

Etiqueta	Entrevistador Iniciais: _____ Data: ___/___/___	Codificação	1ª Digitação	2ª Digitação

IDENTIFICAÇÃO	
1. Numero de identificação do CASANO |__|__|__|__|	IDNumero |__|__|__|__|
CARACTERISTICAS DO DOMICILIO	
2. Tipo de domicilio: [1] Residencial [2] Residencial/Comercial	D2TipoDomic |__|
3. Situação do domicilio: [1] Consentiu [2] Recusou [3] Fechado	D3SituacaoDo |__|
4. Data de construção do domicilio: |__|__|__|__| ano	D4DataDomic |__|__|__|__|
5. Modificações estruturais na casa desde data da coleta: [0] Não [1] Sim	D5ModifEstr |__|
6. Modificações no peridomicilio desde data da coleta: [0] Não [1] Sim	D6Perid |__|
7. Modificações no lixo desde data da coleta: [0] Não [1] Sim	D7Lixo |__|
8. Modificações (fecharam) no esgoto desde data da coleta: [0] Não [1] Sim	D8Esgoto |__|
INSPEÇÃO PERIDOMICILAR	
ANIMAIS DOMESTICOS	
9. Tinha cachorro/s na data da coleta: |__|__| N°	A1CachColet |__|__|
10. Esse/s cachorro/s estão presentes atualmente: |__|__| N°	A2CachPresen |__|__|
11. Cães: |__|__| N°	A3Caes |__|__|
12. Gatos: |__|__| N°	A4Gatos |__|__|
13. Galinhas: |__|__| N°	A5Galinhas |__|__|
14. Outros: |__|__| N° Quais_____	A6Outros |__|__| A7OutrosQuais_____
EXTERIOR DA CASA	
15. Tipo de domicilio: [1] Madeira [2] Alvenaria (N rebocada) [3] Rebocada	E1TipoConstr |__|
16. Vãos no chão: [1] 0 [2] 1-5 [3] 6-10 [4] 11-20 [5] + de 20	E2VaosChao |__|
17. Vãos entre as paredes: [1] 0 [2] 1-5 [3] 6-10 [4] 11-20 [5] + de 20	E3VaosParedes |__|
18. Vãos no telhado: [1] 0 [2] 1-5 [3] 6-10 [4] 11-20 [5] + de 20	E4VaosTeto |__|
PERIDOMICILIO	
19. Escadaria drenante [1] Ausente [2] Integra [3] Danificada	P1EscadDrena |__|
20. Rede de esgoto aberta NG |__|__| .|__|__| x |__|__| .|__|__| m^2	P2LargEsgoto |__|__| .|__|__| m^2 P3CumpEsg |__|__| .|__|__| m^2
21. Tipo de rua de frente do imóvel: [1] Terra [2] Pavimentada	P4TipoRua |__|
22. Transito da rua frente ao imóvel: [1] Alto [2] Meio [3] Baixo	P5TransitRua |__|

23. Área peridomiciliar com chão pavimentado |__|__| .|__|__| x |__|__| .|__|__| m^2	P6AreaPav |__|__| .|__|__| m^2
24. Área do peridomicilio com chão de terra |__|__| .|__|__| x |__|__| .|__|__| m^2	P7AreaTerra |__|__| .|__|__| m^2
25. Ladeira de terra: **0** Não **1** Sim	P8LadeiraTerra |__|
26. Presencia de cerca de arbusto: |__|__| .|__|__| m^2	P9CerArbust |__|__| .|__|__| m^2
27. Presencia de cerca de acumulo: |__|__| .|__|__| m^2	P10CerAcum |__|__| .|__|__| m^2
28. Presencia de cerca de construção: |__|__| .|__|__| m^2	P11CerConst |__|__| .|__|__| m^2
29. Galpões e banheiros externos: |__|__| N°	P12GalpBanhe |__|__|
30. Lixo acessível: **0** Não **1** Sim	P13LixoAcess |__|
31. Alimentos disponíveis: **0** Não **1** Sim	P14AlimDisp |__|
32. Alimentos para animais: **0** Não **1** Sim	P15AlimAnim |__|
33. Água: **1** Não **2** Empoçada **3** Vazamento	P16Agua |__|
34. Material de construção: **0** Não **1** Sim	P17MatConst |__|
35. Entulho: **0** Não **1** Sim	P18Entulho |__|
36. Inservíveis/Objetos abandonados: |__|__|N°	P19InserAban |__|__|
37. Árvores frutíferas: **1** 0 **2** 1-5 **3** 6-10 **4** 11-20 **5** + de 20	P20Frutiferas |__|
38. Plantas ornamentais: **1** 0 **2** 1-5 **3** 6-10 **4** 11-20 **5** + de 20	P21Ornamentais |__|
39. Mato: Largura |__|__| .|__|__| m Cumprimento |__|__| .|__|__| m Altura |__|__| .|__|__| m	P22Mato |__|__|__|.|__|__| m^2
SINAIS DE ROEDORES	
40. Pelagem de roedores (sítios): |__|__| N°	Sp1Pelagem |__|__|
41. Material fecal de *R. norvegicus* (sítios): |__|__| N°	Sp2FezesRn |__|__|
42. Material fecal de *R. rattus* (sítios): |__|__| N°	Sp3FezesRr |__|__|
43. Material fecal de *M. domesticus* (sítios): |__|__| N°	Sp4FezesMd |__|__|
44. Marcas de gordura de *R. norvegicus* (quantas): |__|__| N°	Sp5GorduraRn |__|__|
45. Marcas de gordura de *R. rattus* (quantas): |__|__| N°	Sp6GorduraRr |__|__|
46. Rastros (sítios): |__|__| N°	Sp7Rastros |__|__|
47. Roídas (sítios): |__|__| N°	Sp8Roidas |__|__|
48. Tocas (quantas): |__|__| N°	Sp9Tocas |__|__|
49. Trilhas (quantas): |__|__| N°	Sp10Trilhas |__|__|
INSPEÇÃO EXTERNA AO PERIDOMICÍLIO:	
Nos prédios circundantes são registrados:	
50. Terreno baldio: |__|__| .|__|__| x |__|__| .|__|__| m^2	IE1Baldio |__|__|__|.|__|__| m^2

51. Casa abandonada: **0** Não **1** Sim	IE2CasaAband |__|
52. Ponto de lixo não georreferenciado: |__||__| .|__||__| x |__||__| .|__||__| m^2	IE3LixoNG |__||__|.|__||__| m^2