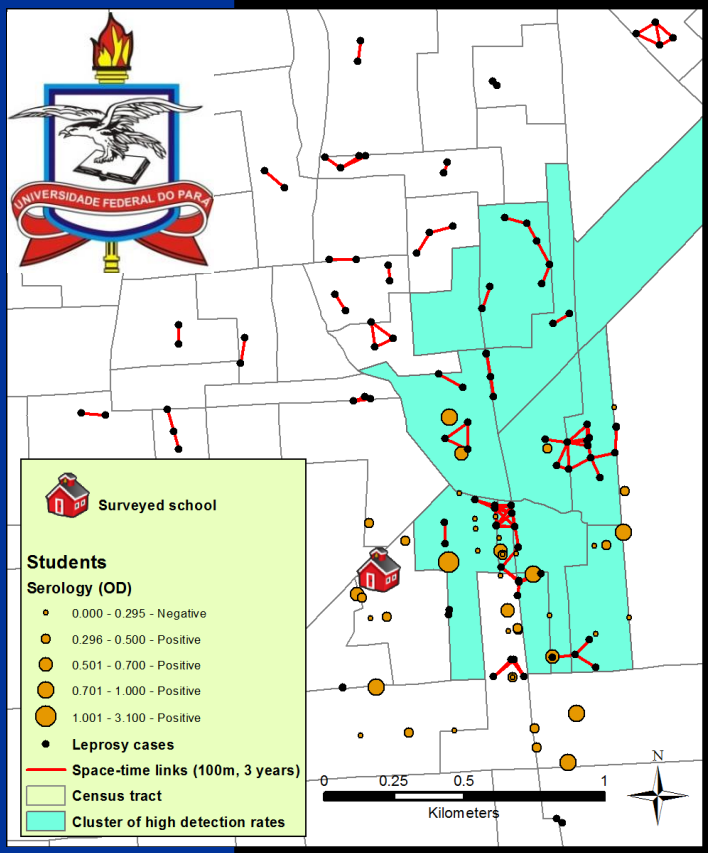
EPIDEMIOLOGIA ESPACIAL E SOROLÓGICA DA HANSENÍASE NO ESTADO DO PARÁ

Tese de doutorado em doenças tropicais

Josafá Gonçalves Barreto



UNIVERSIDADE FEDERAL DO PARÁ

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Tese apresentada ao Programa de Pós-graduação em Doenças Tropicais, do Núcleo de Medicina Tropical da Universidade Federal do Pará, como requisito parcial para a obtenção do grau de Doutor em Patologia das Doenças Tropicais.

Orientador: Prof. Dr. Claudio Guedes Salgado.

Orientador do estágio de doutorado no exterior: Prof. Dr. Uriel Kitron. (*Emory University, Atlanta, GA, USA*).

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“A paixão pela descoberta é cultivada pelo reconhecimento de ser temporariamente ignorante.”

Autor desconhecido!

NOTA SOBRE A FORMATAÇÃO DESTA TESE

Esta tese foi elaborada no formato de agregação de artigos científicos, opção aceita pela RESOLUÇÃO N.º 3.359, DE 14 DE JULHO DE 2005, que institui o regimento geral dos cursos de pós-graduação *stricto sensu* oferecidos pela Universidade Federal do Pará, e também pelo regulamento interno deste Programa de Pós-graduação em Doenças Tropicais.

Neste formato, o documento é composto por um texto integrador (introdução, objetivos e metodologia) seguido dos artigos completos publicados ou submetidos a revistas científicas nacionais ou internacionais, finalizando com as conclusões gerais atingidas pela integração dos artigos.

O texto integrador tem uma referência bibliográfica própria. Neste caso, optei em utilizar as regras de formatação do ICMJE (*International Committee of Medical Journal Editors*) disponíveis em http://www.icmje.org/. Os artigos que compõem os demais capítulos desta tese possuem suas referências bibliográficas formatadas de acordo com as normas das revistas às quais os manuscritos foram submetidos.

RESUMO

Mais de 80.000 casos de hanseníase foram diagnosticados nos últimos 20 anos no Pará e, ainda hoje, com um coeficiente de detecção anual de 50/100.000 habitantes (três vezes superior à média nacional) a doença permanece como um grave problema de saúde pública neste Estado. O objetivo geral deste estudo foi desenvolver um método integrando a epidemiologia espacial e sorológica como ferramenta de combate à hanseníase no Pará. Inicialmente, foram realizadas visitas domiciliares a famílias de pessoas afetadas pela hanseníase, diagnosticadas nos últimos cinco a seis anos, em oito municípios de diferentes regiões do Estado. A equipe de pesquisadores com experiência no manejo da hanseníase, composta por médicos dermatologistas, enfermeiros, fisioterapeutas e técnicos de laboratório, realizou exame clínico dermatoneurológico em 1.945 contatos intradomiciliares de 531 casos notificados e coletou amostra de sangue para pesquisa sorológica de anticorpos IgM anti-PGL-I. Além disso, 1.592 estudantes de 37 escolas públicas do ensino fundamental e médio, com idade entre 6 e 20 anos, também foram selecionados aleatoriamente para serem submetidos à mesma avaliação. As residências dos casos notificados, bem como a dos estudantes incluídos no estudo foram georreferenciadas para a análise da distribuição espacial da hanseníase. Dois anos mais tarde, com base na informação sorológica prévia, a equipe de pesquisadores retornou a dois municípios para reavaliar os indivíduos incluídos no estudo. Adicionalmente, duas novas escolas públicas localizadas em áreas de alto risco de hanseníase, determinadas pela análise da distribuição espacial da doença em um dos municípios, foram selecionadas para avaliar-se a importância da informação geográfica na detecção de casos novos. Na avaliação inicial, 156 (8%) contatos e 63 (4%) estudantes foram diagnosticados como casos novos de hanseníase; 806 (41,4%) contatos e 777 (48,8%) estudantes foram soropositivos para anti-PGL-I. A análise da distribuição espacial dos casos registrados da doença em um dos municípios selecionados indicou que a hanseníase apresenta um padrão heterogêneo, com *clusters* de alta e baixa taxa de detecção anual em áreas específicas da cidade ($p < 0,01$), e que 94,7% dos estudantes examinados residiam a menos de 200 metros de um caso registrado durante os seis anos anteriores ao estudo. No seguimento, a incidência de hanseníase foi significativamente maior entre os indivíduos soropositivos (22,3%) quando comparados aos soronegativos (9.4%) (OR = 2,7; IC95% = 1,29 – 5,87; $p = 0,01$); também foi significativamente mais alta entre moradores de residências com pelo menos um sujeito soropositivo (17,4%), comparada aos de residências sem nenhum morador soropositivo (7,4%) (OR = 2,6; IC95% = 1,18 – 5,91; $p = 0,02$). A seleção de escolas localizadas em áreas de maior risco dentro do município aumentou significativamente a eficiência na detecção de casos novos entre escolares (8,2%), quando comparada aos resultados obtidos em escolas selecionadas aleatoriamente (4%) ($p = 0,04$). Os dados mostram alta taxa de prevalência oculta de hanseníase e de infecção subclínica pelo *M. leprae* no Pará. A epidemiologia espacial e sorológica são ferramentas eficazes para aumentar a detecção precoce de casos novos e deveriam ser utilizadas pelos municípios do Pará para que o Estado possa finalmente alcançar as metas de controle da hanseníase.

Palavras-chave: Hanseníase. Epidemiologia espacial. Sorologia anti-PGL-I. Infecção subclínica. Prevalência oculta.

ABSTRACT

Leprosy remains a severe public health problem in the State of Pará, Brazil. Over 80,000 cases were detected during the last 20 years in Pará, and currently, the annual case detection rate (50/100,000 inhabitants) is three-fold higher than the Brazilian average. The main objective of this study was to develop a method combining anti-PGL-I serology and spatial epidemiology as a tool for reducing the leprosy disease burden in Pará. An initial cross-sectional survey was conducted in eight municipalities of Pará at the residences of people reported to be affected by leprosy during the last five to six years. A group of researchers with experience treating leprosy patients, including dermatologists, nurses, physical therapists and lab technicians, performed a dermatoneurologic clinical examination and collected blood samples to test for anti-PGL-I IgM in 1,945 household contacts (HHC) of the 531 reported cases. Additionally, 1,592 school children (SC), aged 6-20 years, from 37 randomly selected elementary and secondary public schools underwent the same clinical and serologic evaluation. The residential addresses of reported leprosy cases and the residences of the examined SC were georeferenced to determine the spatial distribution pattern of leprosy. Two years later, based on the previous serological data, we returned to two cities to re-examine the same subjects. To evaluate the significance of geographic information in detecting new cases, we also selected two new public schools located in high-risk areas for leprosy. High-risk areas were determined by the spatial analysis of the distribution of cases in one municipality. During the initial survey, 156 (8%) HHC and 63 (4%) SC were diagnosed as new leprosy cases; 806 (41.4%) HHC and 777 (48.8%) SC tested positive for anti-PGL-I. Spatial analysis of one selected municipality demonstrated heterogeneity in the distribution of leprosy cases, with spatial clusters of high and low detection rates in specific regions of the city ($p<0.01$). Additionally, 94.7% of the initially examined SC lived within less than 200 meters of a leprosy case registered during the six years prior to this study. During follow-up, the incidence of leprosy was significantly higher among seropositive individuals (22.3%) when compared to seronegative individuals (9.4%) (OR = 2.7; 95%CI = 1.29 – 5.87; p = 0.01); leprosy rates were also significantly higher among dwellers of residences with at least one seropositive subject (17.4%), compared with dwellers of residences with no seropositive subjects (7.4%) (OR = 2.6; 95%CI = 1.18 – 5.91; $p = 0.02$). Selecting schools located in areas of the city at high-risk of leprosy increased the efficiency of detecting new cases among SC (8.2%) when compared to randomly selected schools (4%) (p = 0.04). The data indicate a high rate of undiagnosed leprosy cases and of subclinical infection with *M. leprae* in the State of Pará. Anti-PGL-I serology and spatial epidemiology are effective tools to increase the early detection of new cases, and these methods should be used by the municipalities of Pará to help reach leprosy control targets.

Keywords: Leprosy. Spatial epidemiology. Anti-PGL-I serology. Subclinical infection. Hidden prevalence.

LISTA DE ABREVIATURAS

BCG-ID	Bacilo Calmette-Guérin – Intradérmica
BI	Bacilloscopic index
BSA	Bovine serum albumin
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEP-	Comitê de ética em pesquisa/Instituto de Ciências da Saúde/Universidade
ICS/UFPA	Federal do Pará
CGHDE	Coordenação Geral de Hanseníase e Doenças em Eliminação
CI	Confidence interval
CNPQ	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DD	Dimorfa-dimorfa
DEVIT	Departamento de Vigilância das Doenças Transmissíveis
DG	Disability grading
DO	Densidade optica
DT	Dimorfa-tuberculóide
DV	Dimorfa-virchowiana
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FAPESPA	Fundação de Amparo à Pesquisa do Estado do Pará
GIS	Geographic information system
GPS	Global Positioning System
HC or HHC	Household contact
HCSDL	Household contact of student diagnosed with leprosy
HIV/AIDS	Vírus da imunodeficiência humana/Síndrome da imunodeficiência adquirida
HSA	Human serum albumin
I	Indeterminada
IB	Índice baciloscópico
IBGE	Instituto Brasileiro de Geografia e Estatística
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IM	Índice morfológico
IQR	Interquartile range

LID-1	Leprosy Infectious Disease Research Institute Diagnostic-1
LISA	Local indicator of spatial association
LPs	Leprosy patients
MALTALEP	The Order of Malta grants for leprosy
MB	Multibacilar
MDT	Multidrug therapy
MS/SVS	Ministério da Saúde/Secretaria de Vigilância em Saúde
ND	Natural disaccharide
ND-O-BSA	Natural disaccharide-octyl- bovine serum albumin
ND-O-HSA	Natural disaccharide-octyl- human serum albumin
NNH	Number needed to harm
NT	Natural trisaccharide
NT-P-BSA	Natural trisaccharide-propyl- bovine serum albumin
OD	Optical Density
OMS	Organização Mundial da Saúde
OPD	o-Phenylenediamine dihydrochloride
OR	Odds ratio
PA	Pará
PAL	People affected by leprosy
PB	Paucibacilar
PBS	Phosphate buffered saline
PGL-I	Phenolic glycolipid I
pH	Potential hydrogen
PIBIC	Programa Institucional de Bolsas de Iniciação Científica
PNCH	Programa Nacional de Controle da hanseníase
PPD	Purified protein derivative
PPUL	Prevalence of previously undiagnosed leprosy
PQT	Poliquimioterapia
ROC	Receiver operating characteristic
RR	Relative risk
RT	Room temperature
SC	School children
SD	Standard deviation

SEB	Spatially empirical Bayes
SESPA	Secretaria Executiva de Saúde Pública do Estado do Pará
SIG	Sistemas de informação geográfica
SINAN	Sistema de Informação de Agravos de Notificação
SIRGAS	Sistema de Referência Geocêntrico para as Américas
SVS-MS	Secretaria de Vigilância em Saúde - Ministério da Saúde
T	Tuberculóide
T1	First evaluation
T2	Second evaluation (two years later)
UFPA	Universidade Federal do Pará
UREMC	Unidade de Referência Especializada em Dermatologia Sanitária Dr. Marcello Candia
USA	United States of America
UTM	Universal Transverse Mercator
V	Virchowiana
WHO	World Health Organization

SUMÁRIO

CAPÍTULO 1

INTRODUÇÃO

1.1. Aspectos gerais sobre a hanseníase

A hanseníase é uma doença infectocontagiosa crônica causada pelo *Mycobacterium leprae*, um bacilo intracelular obrigatório com predileção pela célula de Schwann nos nervos periféricos e macrófagos no tecido cutâneo. O *M. leprae* foi a primeira bactéria identificada como causadora de doença em humanos, sendo descrita pela primeira vez em 1873 pelo médico norueguês Armauer Hansen, razão pela qual é denominada, também, bacilo de Hansen (1).

O termo hanseníase é utilizado no Brasil desde a década de setenta e tornou-se de uso obrigatório a partir de 1995, em substituição a antiga denominação lepra, por meio da Lei Federal número 9.010, em uma tentativa de diminuir o estigma da doença que tem causado sofrimento à humanidade desde a antiguidade (2).

A hanseníase existiu em todos os continentes e deixou uma terrível imagem de mutilação, rejeição e exclusão social na história e na memória da humanidade. Tem provocado medo nas pessoas por milênios, desde os primeiros relatos em civilizações antigas da China, Egito e Índia. O número cumulativo total de indivíduos que sofreram com o curso crônico da doença nunca poderá ser calculado (3).

O *M. leprae* não é cultivável em meios de cultura, o que constitui a principal dificuldade da pesquisa em hanseníase. Seu tempo de reprodução é muito lento (12 a 14 dias) e quantidades suficientes do bacilo somente foram obtidas para análises biológicas e imunológicas quando foi descoberto que tatus (*Dasypus novemcinctus*) poderiam ser infectados (4, 5). A viabilidade do bacilo no meio ambiente externo ao corpo humano parece ser influenciada pela temperatura, humidade e luminosidade, variando de 46 dias no solo úmido a cinco meses em ambiente sombreado (6).

1.2. Transmissão

O modo de infecção pelo *M. leprae* ainda permanece desconhecido, apesar de vários mecanismos terem sido propostos (7-10). Uma vez que o homem é encarado como um dos principais reservatórios do *M. leprae*, a entrada e saída de bacilos através das vias aéreas superiores e sua transmissão direta de pessoa para pessoa ainda é considerada como a principal via de contágio (11, 12).

Acredita-se que os pacientes multibacilares (MB) sem tratamento são as principais fontes de infecção e seus contatos intradomiciliares constituem o principal grupo de risco para o desenvolvimento da doença (13). Entretanto, em regiões de alta endemicidade, grande parte da população estaria exposta ao *M. leprae* (14), porém apenas uma pequena proporção, estimada em 10% dos sujeitos infectados, desenvolveria a doença devido à alta infectividade, mas baixa patogenicidade do bacilo (15). Outros fatores como a genética do hospedeiro, situação socioeconômica, fome, baixa escolaridade e falta de saneamento básico possuem papel importante na infecção e no desenvolvimento da doença (16-18).

A hanseníase possui um longo período de incubação, com média de 3 a 5 anos, podendo se estender por décadas, como descrito recentemente em um caso envolvendo um chimpanzé (*Pan troglodytes*), cujo período entre a infecção e o surgimento das manifestações clínicas da doença foi de 30 anos (19). A importância na cadeia epidemiológica de pessoas saudáveis portadoras do bacilo vem sendo discutida (20). Neste aspecto, o ambiente domiciliar é apontado como meio facilitador no processo de transmissão, prolongando o contato entre familiares saudáveis e infectados, aumentando de cinco a dez vezes as chances de infecção (21, 22).

Tem sido demonstrado que, além dos contatos intradomiciliares, os vizinhos próximos de um caso de hanseníase e os contatos sociais (na escola, trabalho, igreja, etc.) também apresentam maior risco de adoecimento quando comparados com a população em geral (22-24).

Apesar do contato íntimo e prolongado ser considerado o principal modo de difusão do *M. leprae*, alguns casos não conseguem ser relacionados ao contato direto e/ou intercorrente com pacientes portadores de hanseníase. Tal fato conduz o foco a novas possibilidades de transmissão, como água, solo, plantas e diferentes espécies de animais incluindo ameba, insetos, peixe e tatus (7, 25). Entretanto, a verificação experimental destas fontes alternativas é difícil, uma vez que o *M. leprae* não é cultivado em meios artificiais.

A ocorrência de casos em menores de 15 anos de idade é um importante indicador de transmissão recente e da existência de focos ativos de infecção não diagnosticados na comunidade onde elas vivem (26) e a detecção precoce de casos nesta faixa etária é uma das prioridades dos planos de controle da hanseníase (27, 28).

1.3. Epidemiologia

A prevalência mundial da hanseníase, registrada no final do primeiro trimestre de 2013, foi de 189.018 casos e o número de casos novos registrados durante 2012 foi de 232.857. Dentre estas notificações, 137.410 (59%) casos foram classificados como MB; 21.349 (9,2%) foram pessoas menores de 14 anos de idade e 14.409 (6,2%) já apresentavam grau 2 de incapacidade física no momento do diagnóstico. Atualmente apenas 16 países reportam mais de 1.000 casos novos anualmente (Bangladesh, Brasil, China, Costa do Marfim, Congo, Etiópia, Índia, Indonésia, Madagascar, Mianmar, Nepal, Nigéria, Filipinas, Sudão do Sul, Sri Lanka e Tanzânia) (29).

Desde 1985, após a implementação da poliquimioterapia (PQT), mais de 16 milhões de pessoas foram curadas da hanseníase. No entanto, mesmo com a evolução do tratamento, a doença permanece como um problema de saúde pública no Brasil, onde não se conseguiu alcançar a meta proposta pela Organização Mundial de Saúde (OMS) para o ano de 2005 de até 1 caso/10.000 habitantes. Com 33.303 novos casos detectados em 2012, o Brasil ocupa o primeiro lugar nas Américas e o segundo lugar mundial, ficando atrás somente da Índia que registrou 134.752 casos novos naquele ano (29). A Figura 1 ilustra a distribuição mundial da hanseníase de acordo com o coeficiente de detecção anual registrado no início de 2012.

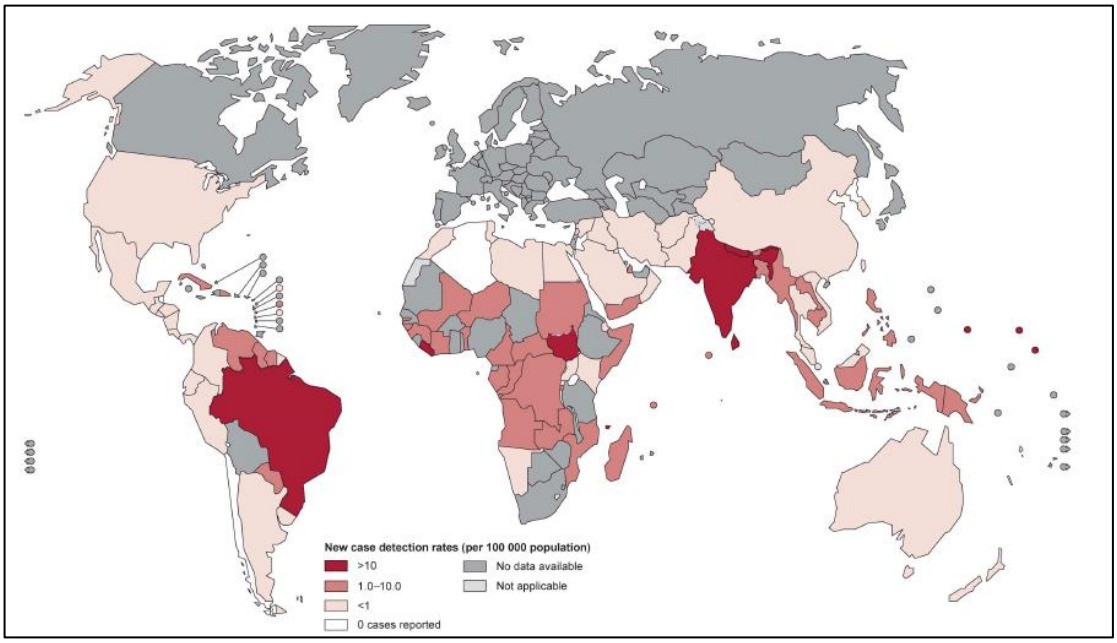

Figura 1: Distribuição mundial da hanseníase registrada em janeiro de 2012. O Brasil possui um dos maiores coeficientes de detecção anual do mundo (17,2/100.000 habitantes). Fonte: http://www.who.int/lep/situation/Leprosy_DR_2011.pdf

A distribuição da hanseníase no Brasil não apresenta uniformidade. A tendência da detecção de casos novos é decrescente no país, mas nota-se, ainda, alta incidência nos Estados das regiões Norte, Centro-Oeste e Nordeste, quando comparados aos das regiões Sul e Sudeste. Alguns Estados, tais como Rio Grande do Sul, Santa Catarina e Paraná já alcançaram a meta de controle da doença. Contudo, o Estado do Pará, com 3.912 casos novos diagnosticados em 2012, resultando em um coeficiente de detecção anual de 50/100.000, é considerado hiperendêmico e, atualmente, faz parte de uma das regiões com a maior carga da doença no mundo (30, 31).

Dezenas de municípios do Estado do Pará estão inseridos nas dez áreas de maior risco de hanseníase no Brasil, notadamente os municípios das regiões Sul e Sudeste do Estado, inseridos no cluster 1, o de maior risco de detecção de casos no país, como demonstrado pelo trabalho de Penna *et al.* (32). Municípios das regiões central e oeste do Pará estão incluídos no cluster 7 (Figura 2).

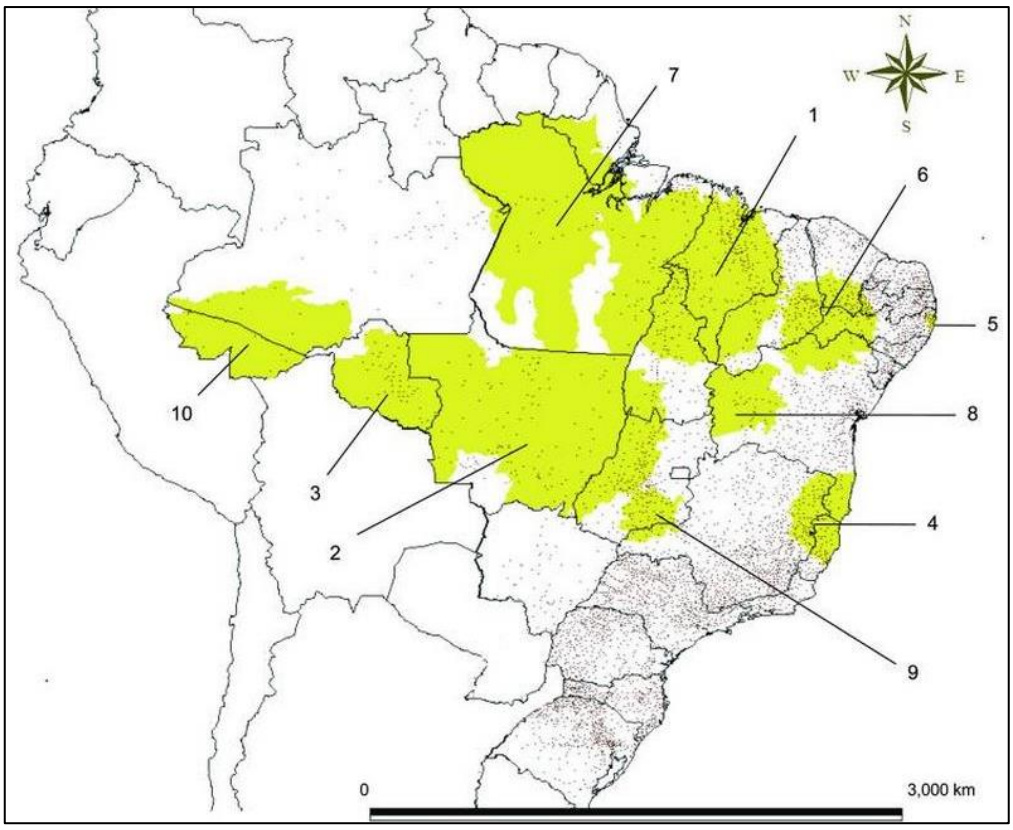

Figura 2: Os 10 principais clusters de hanseníase no Brasil. A distribuição da doença no Brasil não é uniforme.
Fonte: Penna *et al.*Emerg Infect Dis, v. 15, n. 4, p. 650-2, Apr, 2009.

Dados oficiais da Coordenação Geral de Hanseníase e Doenças em Eliminação do Ministério da Saúde do Brasil (CGHDE) apontam que a hanseníase é um problema histórico

no Pará, onde mais de 88.000 casos foram diagnosticados entre os anos de 1990 e 2010 (33). Atualmente, o coeficiente de detecção no Pará é aproximadamente 3 vezes superior a média nacional (Figura 3).

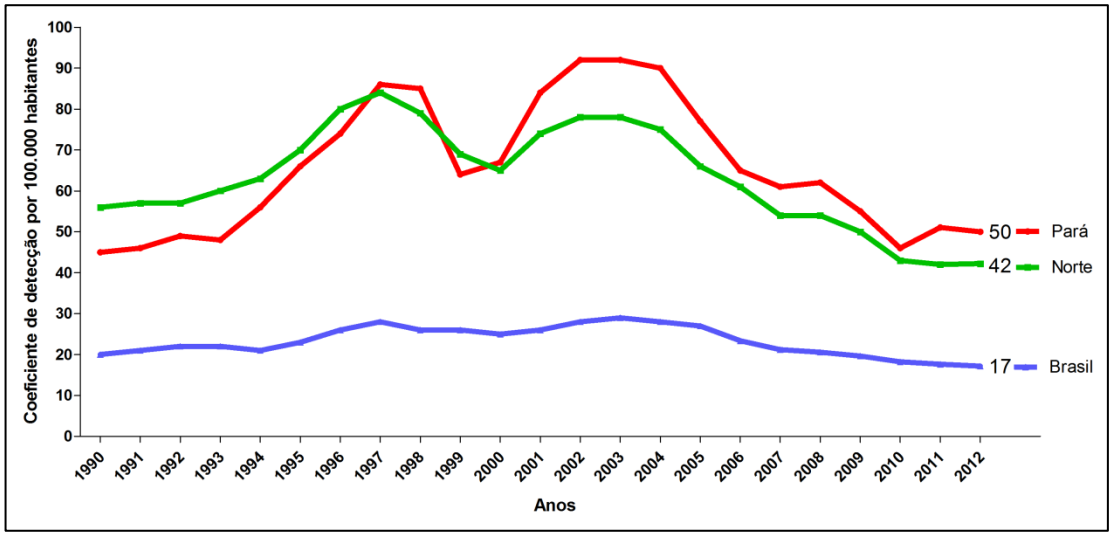

Figura 3: Coeficiente de detecção de hanseníase na população geral, Pará, Região Norte e Brasil, 1990 a 2012.
Fonte: CGHDE/DEVIT/SVS-MS

Também existem fortes evidências de que há focos ativos de transmissão na região, dado que o Estado é hiperendêmico entre menores de 15 anos de idade, com uma taxa de 15,3/100.000 habitantes nessa faixa etária, bem acima da média nacional de 4,8/100.000 (34). Adicionalmente, estima-se que a prevalência não diagnosticada na população em geral, em regiões de alta endemicidade, seria equivalente a seis vezes a prevalência registrada (35).

A intensa migração populacional para o Norte do Brasil, intensificada após a década de 1970 com a implantação de grandes projetos desenvolvimentistas na região, tem sido apontada como um fator complicador para o controle da endemia, aumentando a pressão sobre uma já precária infraestrutura de serviços públicos (36-38).

1.4. Manifestações clínicas

A hanseníase é caracterizada por uma grande diversidade no seu curso clínico, variando de uma doença com poucos bacilos, número reduzido de lesões e nervos periféricos acometidos, a uma doença com grande carga bacilar presente nas diversas lesões infiltrativas, progressivas e difusas da pele e nervos periféricos, mucosas das vias aéreas superiores, olhos, testículos, podendo afetar, ainda, os linfonodos, o fígado e o baço (39).

A intensidade das manifestações clínicas é determinada pela resposta imune do hospedeiro ao *M. leprae*. Em geral, as manchas hipocrômicas hipoestésicas ou anestésicas (tátil, térmica e dolorosa) são os primeiros sinais da doença. Estas lesões poderão curar-se espontaneamente ou evoluir para uma das formas mais graves da doença. Com a evolução da enfermidade, novas lesões de pele podem surgir, variando em quantidade, forma, aparência e coloração, mas sempre com a alteração de sensibilidade sendo a característica patognomônica da hanseníase (40).

As repercussões clínicas que mais preocupam os profissionais da saúde e os pacientes são decorrentes da neuropatia periférica, desencadeada pela presença do bacilo nas terminações nervosas da pele e em grandes troncos nervosos (41). A lesão neural leva a alterações sensitivas, motoras e autonômicas nos membros inferiores, superiores, face e tronco. Sucessivas lesões traumáticas nos pés e nas mãos levam ao surgimento das úlceras hansênicas, uma das sequelas mais estigmatizantes da doença. Sua presença é bastante incapacitante para o indivíduo, tanto no aspecto físico quanto no âmbito psicossocial, podendo levar a deformação e/ou amputação do membro afetado (42).

Durante a evolução da doença, podem ocorrer surtos abruptos de agudização, resultando em reações imunológicas denominadas reações hansênicas. Estes episódios inflamatórios se intercalam no curso crônico da hanseníase, podendo surgir antes do diagnóstico, durante o tratamento e mesmo após anos de alta por cura. Os estados reacionais são classificados em reação do tipo 1 (reversa) e reação do tipo 2 ou eritema nodoso hansênico. Eles devem ser prontamente diagnosticados e tratados, pois são a principal causa dos danos neurais e incapacidades na hanseníase (43).

1.5. Diagnóstico

O diagnóstico de caso de hanseníase é essencialmente clínico e epidemiológico, realizado por meio da análise da história e condições de vida do paciente e do exame dermatoneurológico, para identificar lesões ou áreas de pele com alteração de sensibilidade e/ou comprometimento de nervos periféricos (sensitivo, motor e/ou autonômico) (44).

O Ministério da Saúde define como caso de hanseníase para tratamento, quando um ou mais dos seguintes achados cardinais encontram-se presentes: **[1]** lesão(ões) e/ou área(s) da pele com alteração de sensibilidade; **[2]** acometimento de nervo(s) periférico(s), com ou sem

espessamento, associado a alterações sensitivas e/ou motoras e/ou autonômicas; **[3]** baciloscopia positiva de esfregaço intradérmico para *M. leprae* (45).

Não existe nenhum exame laboratorial capaz de diagnosticar todas as formas clínicas de hanseníase. A baciloscopia, apesar de ser considerada o padrão ouro entre os testes laboratoriais para hanseníase, sempre será negativa nas formas paucibacilares (PB) da doença. Em outras palavras, uma baciloscopia negativa não exclui o diagnóstico de hanseníase (45).

Quando positiva, a baciloscopia permite identificar a carga bacilar do paciente, expressada pelo índice baciloscópico (IB), bem como o índice morfológico (IM), um indicador da viabilidade dos bacilos encontrados na amostra. O IB representa o número de bacilos encontrado em um campo microscópico, em uma escala logarítmica, de 1+ (1 a 10 bacilos em 100 campos examinados) a 6+ (mais de 1000 bacilos, em média, em cada campo examinado). O raspado dérmico deve ser coletado em quatro sítios, incluindo lóbulos auriculares, cotovelo e lesão cutânea, quando presente (46).

Outros exames complementares podem auxiliar no diagnóstico diferencial e na classificação dos casos, tais como a histopatologia de biópsia de pele ou nervo periférico e exames eletrofisiológicos, especialmente úteis nos casos sem manifestações dermatológicas, como no exemplo da hanseníase primariamente neural que corresponde a aproximadamente 10% do total de casos (47).

1.6. Classificação

As classificações mais usadas são as de Madri (Congresso Internacional, 1953), a de Ridley e Jopling de 1966, e a classificação operacional da OMS. A classificação de Madri considera dois polos estáveis e opostos (virchowiano e tuberculóide) e dois grupos instáveis (indeterminado e dimorfo), que caminhariam para um dos polos na evolução natural da doença (48).

A classificação proposta por Ridley e Jopling (49), bastante utilizada na pesquisa científica, leva em consideração a imunidade dentro de um espectro de resistência do hospedeiro. São descritas as formas tuberculóide (T), onde o hospedeiro apresenta maior grau de imunidade celular contra o bacilo; os casos borderline ou dimorfos que são subdivididos em dimorfo-tuberculóide (DT), dimorfo-dimorfo (DD) e dimorfo-virchowiano (DV); e virchowiano (V), onde a resposta imune celular do hospedeiro é menor, ou mesmo ausente. A

resposta humoral do hospedeiro é inversamente proporcional à resposta celular, estando bastante exacerbada no polo V e discreta no polo T da doença (50).

Visando simplificar a classificação da doença para fins de tratamento com a PQT, a OMS rotula operacionalmente os casos em paucibacilares (PB), quando apresentam até 5 lesões de pele sem infiltração, e em multibacilares (MB) quando apresentam mais de 5 lesões ou baciloscopia positiva (27). As formas I, da classificação de Madri, e T da classificação de Ridley e Jopling estão entre as PB, enquanto que as formas DT, DD, DV e V são classificadas como MB (44).

1.7. Tratamento e medidas de controle

O diagnóstico precoce, o tratamento medicamentoso adequado por meio da PQT/OMS, a prevenção e tratamento de incapacidades físicas, e a vigilância dos contatos intradomiciliares constituem a base dos programas de controle da hanseníase (27).

O tratamento com a PQT/OMS, constituído pela rifampicina, dapsona e clofazimina tem se mostrado eficiente na cura da infecção, apresentando baixos índices de recidiva (0 a 7,7%) (39). Os casos PB são tratados com um esquema padronizado de 6 doses (6 meses de tratamento) incluindo a rifampicina e a dapsona. Já os casos MB são tratados com 12 doses (12 meses de tratamento), com um esquema que inclui a clofazimina, além da rifampicina e dapsona (45).

Todos os pacientes devem ser orientados quanto às medidas de autocuidados incluindo a auto inspeção diária dos olhos, nariz, mãos e pés. Calçados e utensílios domésticos podem ser adaptados para aumentar a proteção de membros com perda de sensibilidade. No caso de perda de força muscular, diversos exercícios podem ser realizados com objetivo de recuperar a função motora.

O exame e a vigilância de contatos intradomiciliares são indispensáveis para o diagnóstico precoce, uma vez que este é o principal grupo de risco para a doença (21). Recomenda-se que esta estratégia seja implementada de forma ativa, aumentando a taxa de contatos examinados. A investigação consiste no exame dermatoneurológico de todos os contatos intradomiciliares dos casos novos detectados, independentemente da classificação operacional, e do repasse de orientações sobre período de incubação, transmissão e sinais e sintomas precoces da hanseníase (45).

Para os contatos que não apresentam sinais e sintomas de hanseníase, o Ministério da Saúde do Brasil preconiza a vacinação com BCG-ID (45), independentemente de serem contatos de casos PB ou MB, uma vez que existem evidências de que a vacina, mesmo não sendo específica para a hanseníase, confere 56% de proteção contra a doença (51).

1.8. Sorologia em hanseníase

A descoberta na década de 1970 de que o *M. leprae* poderia ser cultivado *in vivo,* utilizando tatus (*Dasypus novemcinctus*) (5), possibilitou pela primeira vez uma quantidade suficiente de bacilos para o estudo da biologia deste patógeno, resultando em significativos avanços desde então. Um dos mais importantes foi a identificação do glicolipídeo fenólico I (PGL-I) por Brennan e Barrow (52) na década de 1980, uma molécula espécie-específica abundante na parede celular do *M. leprae e* com alto poder imunogênico.

O PGL-I evoca intensa produção de anticorpos da classe IgM, especialmente entre os pacientes do polo virchowiano. A imunogenicidade da molécula de PGL-I é largamente atribuída ao seu componente *3,6-di-O-methyl-β-D-glucosyl* presente na porção terminal do seu trissacarídeo (Figura 4). Novas glicoproteínas semissintéticas contendo o terminal dissacarídeo (ND) ou o trissacarídeo (NT) sintético inteiro do PGL-I, conjugados a albumina do soro bovino (BSA) ou humano (HSA) por meio de uma proteína de ligação (usualmente *octyl* ou *phenyl*) foram desenvolvidas (ND-O-BSA, ND-O-HSA, NT-P-BSA, entre outras) com objetivo de facilitar a produção do antígeno e torná-lo hidrossolúvel, disponibilizando-o a pesquisadores de vários países e permitindo o desenvolvimento de testes sorológicos rápidos (53).

Vários estudos demonstraram que a sorologia poderia ser utilizada para detectar anticorpos anti-PGL-I e a sua titulação poderia ser útil na classificação dos pacientes, monitoramento dos casos, identificação do risco de recidivas e identificação de contatos intradomiciliares com maior risco de desenvolver a hanseníase (50, 54-56).

A sorologia anti-PGL-I apresenta de moderada a boa correlação com o índice baciloscópico do paciente (57, 58), entretanto não possui a sensibilidade necessária para ser utilizada como um teste diagnóstico. Aproximadamente 90% dos pacientes MB apresentam sorologia positiva, enquanto que usualmente apenas 20% a 40% dos casos PB são positivos para anti-PGL-I (50, 54).

A titulação dos anticorpos também é significativamente mais elevada entre os pacientes MB, com progressivo e consistente aumento até o polo virchowiano (53). É possível observar significativa diminuição dos níveis de anti-PGL-I após o início da PQT/OMS, sendo a velocidade da queda bastante variável (25% a 50% por ano) e diretamente relacionada com a carga bacilar do paciente (56, 59, 60). Foi demonstrado que uma repentina elevação nos níveis de anticorpos após o período de tratamento poderia ser preditivo de um episódio de recidiva da doença (61, 62).

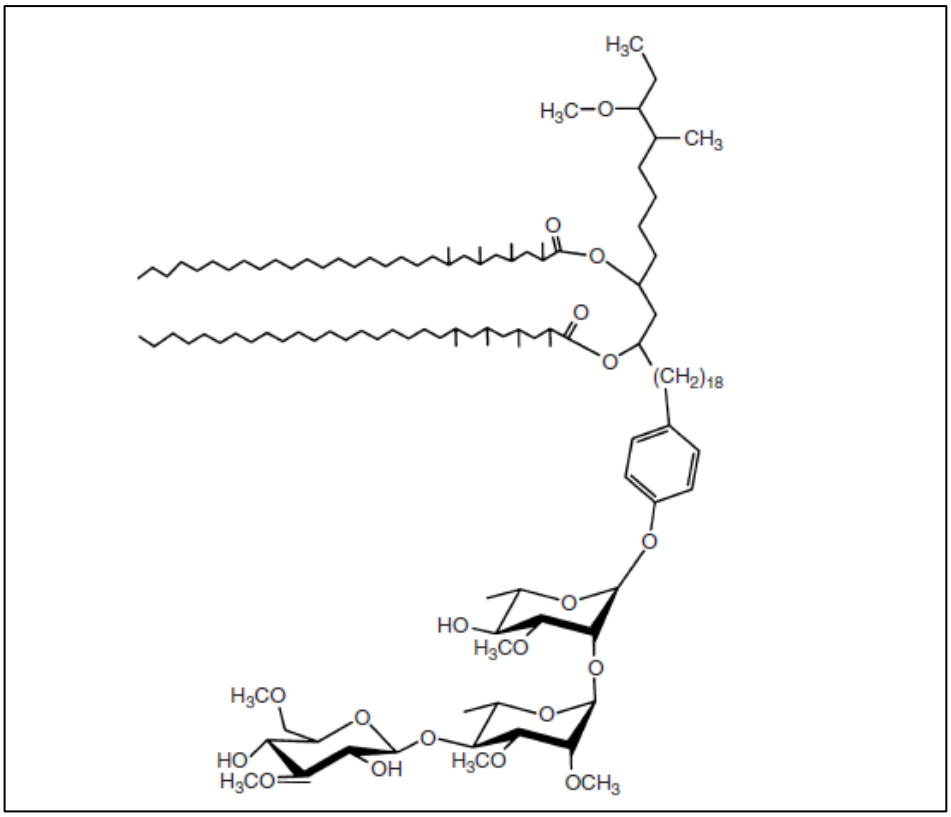

Figura 4: Estrutura química da molécula de PGL-I.
Fonte: Spencer and Brennan. Lepr Rev (2011) 82, 344 – 357.

O anti-PGL-I tem sido interpretado como um biomarcador de infecção subclínica entre indivíduos sem sinais e sintomas de hanseníase (63, 64). Uma sorologia positiva está associada a um risco 8,6 vezes maior de adoecimento entre contatos intradomiciliares e 4,4 entre não-contatos, quando comparados com a população em geral (65). A soroprevalência entre contatos varia usualmente entre 10 e 20% (54, 66), com algumas evidências de que seria maior entre comunicantes de pacientes MB (67, 68). Foi observado que com o tratamento do caso índice, ocorre uma diminuição dos níveis de anticorpos entre os seus contatos intradomiciliares, indicando uma redução da carga de exposição ao bacilo (56).

Além disso, alguns estudos apontam que a soroprevalência na população em geral também parece indicar o nível de endemicidade na comunidade, variando de 0,7% em áreas não endêmicas a 27% em áreas endêmicas (69-73). Desta forma, a soroprevalência poderia ser um indicador apropriado para a carga da doença em uma determinada área. Entretanto, esses dados indicam que a infecção subclínica é muito comum em áreas endêmicas, mas que apenas uma pequena parcela dos sujeitos infectados evoluirá para a doença clínica (74).

O PGL-I é certamente o antígeno mais estudado na pesquisa em hanseníase, entretanto diversos outros têm sido investigados, incluindo mais de uma centena de proteínas recombinantes do *M. leprae*, na tentativa de se identificar um candidato a um teste suficientemente sensível e específico para o diagnóstico de todas as formas clínicas da doença (75).

Recentemente, o LID-1 (*Leprosy Infectious Disease Research Institute Diagnostic-1*), uma proteína sintética originada da fusão das proteínas ML0405 e ML2331, mostrou uma boa reatividade com amostras selecionadas de pacientes MB (76, 77). Também foi descrito que altos níveis de anticorpos IgG anti-LID-1 seriam capazes de diagnosticar a hanseníase de 6 a 8 meses antes do início de suas manifestações clínicas (76, 78). Entretanto, os dados são provenientes de pequenas amostras e não existem estudos sobre a reatividade do LID-1 em estudos populacionais em áreas endêmicas e, assim como com o PGL-I, pacientes PB apresentam baixa reatividade contra esta neoproteína (aproximadamente 20% de soropositividade) (76), impedindo o seu uso como teste diagnóstico.

O ELISA (*enzyme-linked immunosorbent assay*) é a técnica mais utilizada na sorologia em hanseníase. Entretanto, a necessidade de recursos humanos capacitados, de equipamentos e suprimentos específicos limita a sua implementação na maioria das áreas com alta carga da doença no mundo. Diferenças nos protocolos utilizados por diversos grupos de pesquisa também prejudicam a comparação dos resultados obtidos, e podem explicar parcialmente as divergências observadas na literatura. Na tentativa de facilitar o acesso à informação sorológica aos profissionais de saúde atuantes em comunidades mais distantes, de forma rápida, simples, economicamente viável e padronizada, alguns testes sorológicos rápidos foram desenvolvidos.

O teste rápido ML-Dipstick usa o antígeno dissacarídeo natural do PGL-I conjugado com a albina do soro bovino (DBSA) imobilizado em uma fita de nitrocelulose. Este teste imunocromatográfico detecta anticorpos específicos da classe IgM e apresentou 97.2% de

concordância com o ELISA anti-PGL-I (Kappa = 0,92) (79). Segundo os autores, o teste poderia contribuir na correta classificação dos pacientes para fins de tratamento com a PQT/OMS (80).

Outro exemplo de teste rápido é o ML-Flow, cujo antígeno é o NT-P-BSA. Ele também detecta anticorpos da classe IgM e apresentou concordância de 91% (Kappa = 0,77) com o ELISA anti-PGL-I (81). Segundo os autores, o teste também poderia ser utilizado para auxiliar na classificação do pacientes, além de identificar contatos com alto risco de desenvolver a hanseníase. Tanto o ML-Flow quando o ML-Dipstick nunca foram recomendados para uso em larga escala no combate a endemia.

Mais recentemente, uma empresa sediada no Brasil (*Orange Life*) lançou um novo teste rápido, cujo antígeno é o resultado da fusão do NDO com o LID-1 (NDO-LID®). Este equipamento, diferentemente dos anteriores, detecta anticorpos das classes IgM e IgG. No único trabalho publicado até a presente data (82), os autores afirmam que este teste apresenta 90,9% de concordância com o ELISA anti-PGL-I (Kappa = 0,8), e até mesmo superaria sua capacidade de detectar casos (19 foram positivos no teste rápido e negativos no ELISA), podendo ser utilizado como um teste diagnóstico e prognóstico. Entretanto, as evidências disponíveis ainda não são suficientes para a ampla recomendação do uso deste teste rápido dentro do programa de controle da hanseníase, devido a escassez de dados em populações de diferentes áreas endêmicas.

1.9. Epidemiologia espacial

Epidemiologia espacial é a descrição e análise das variações geográficas do estado de saúde e doença das populações, correlacionada a fatores de risco demográficos, ambientais, comportamentais, socioeconômicos, genéticos e infecciosos (83). O lugar, ou espaço onde as pessoas vivem e trabalham, há tempos tem sido reconhecido como um componente essencial na epidemiologia (84).

O trabalho pioneiro do médico Britânico John Snow sobre a epidemia de cólera na cidade de Londres em 1854 não somente inaugurou o que hoje chamamos de epidemiologia, mas também é o primeiro exemplo da importância dos mapas para a saúde pública. Ele registrou e mapeou as mortes causadas por cólera, percebendo assim que havia uma concentração de óbitos entre residentes que utilizavam a água proveniente de uma bomba localizada na rua Broad (Figura 5). Snow então conseguiu convencer autoridades locais a

removerem o braço daquela bomba específica, impedindo o consumo de sua água e, consequentemente, diminuindo drasticamente a ocorrência de novos casos (85, 86). Tudo isso foi realizado quase 30 anos antes da descoberta do agente causal da cólera, o *Vibrio cholerae.*

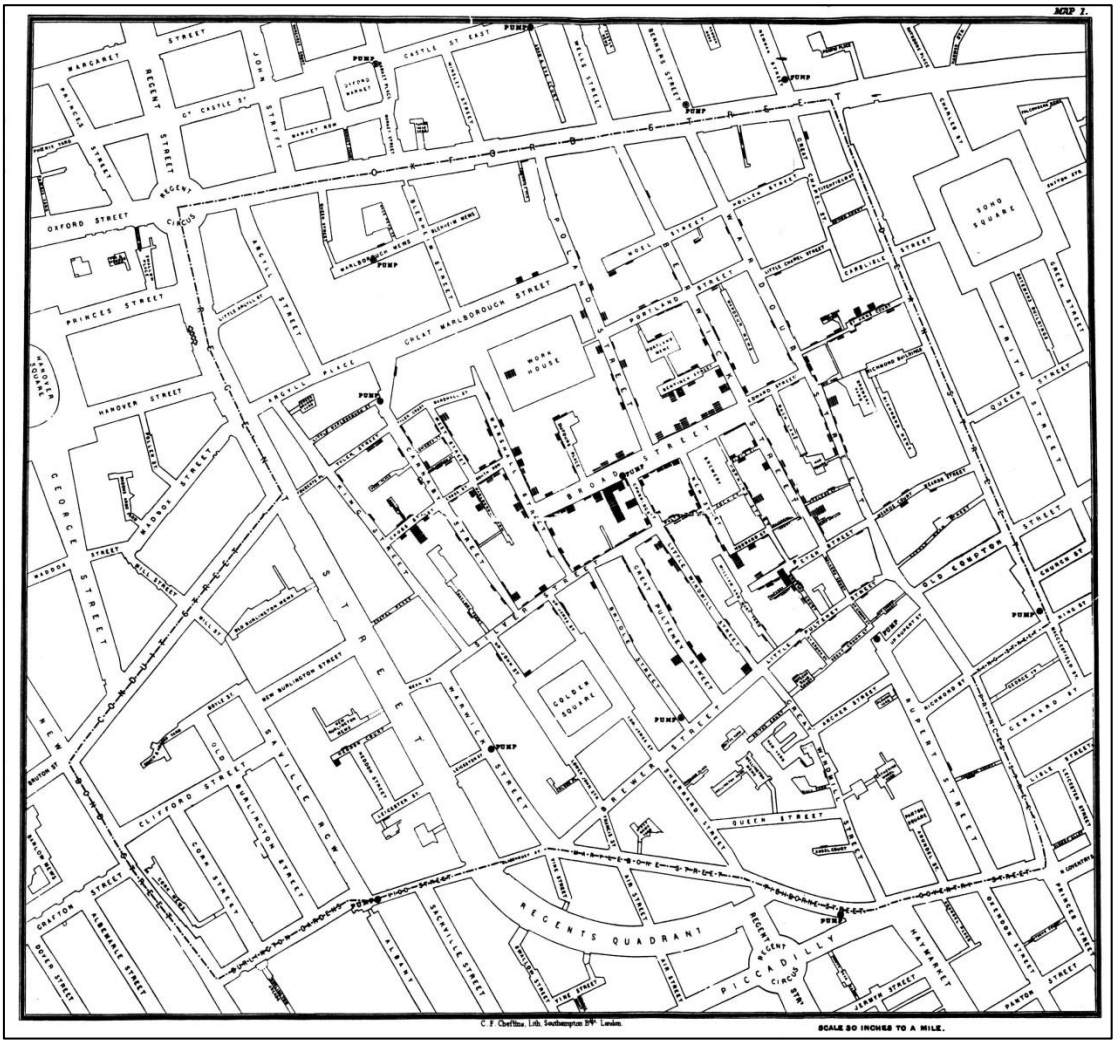

Figura 5: Distribuição espacial das mortes por cólera em Londres, 1854. Mapa original elaborado pelo médico John Snow, considerado um dos "pais da epidemiologia".
Fonte: Snow, John. On the Mode of Communication of Cholera, 2nd Ed, John Churchill, London, England, 1855.

O interesse pela epidemiologia espacial tem crescido nos últimos 20 anos, aumentando sua complexidade, sofisticação e utilidade. O desenvolvimento do sistema de posicionamento global (GPS) entre as décadas de 1970 e 1990 (87), os recentes avanços na capacidade de processamento dos computadores pessoais, nos sistemas de informação geográfica (SIG) e na disponibilização de dados espaciais, ampliaram o acesso destas ferramentas a um grande número de pesquisadores, permitindo o mapeamento com precisão e acurácia de inúmeras variáveis de interesse epidemiológico, criando oportunidades para melhorar o entendimento da dinâmica de diversas doenças em nível internacional, nacional,

regional e local (88). Várias revistas científicas especializadas no tema foram lançadas nos últimos anos, tais como: **[1]** *Spatial and Spatio-temporal Epidemiology;* **[2]** *International Journal of Health Geographics;* **[3]** *Health & Place;* **[4]** *Geospatial Health* e **[5]** *Social Science & Medicine. Part D: Medical Geography*.

Os SIG's têm sido aplicados na pesquisa, prevenção e controle de várias doenças infecciosas, tais como a malária, tuberculose e HIV/AIDS (89). A OMS estimula a utilização de SIG para análises geoespaciais do comportamento da endemia hansênica, com objetivo de identificar padrões de distribuição espacial e temporal dos casos detectados, fornecendo uma análise cartográfica dos indicadores epidemiológicos ao longo do tempo, indicando onde existe a necessidade de implementar esforços extras para o combate a endemia (90).

A identificação da distribuição espacial dos casos, bem como da infecção subclínica pelo *M. leprae*, fornece uma visão privilegiada, facilitando a realização de medidas de combate focadas em regiões específicas, onde o problema pode estar agrupado em forma de *clusters* (aglomerados) (91).

No primeiro estudo utilizando SIG associado a dados clínicos e epidemiológicos em hanseníase, Bakker *et al.* (69) detectaram a formação de *clusters* de soropositividade para anticorpo anti-PGL-I entre pessoas que moravam próximas a pacientes soropositivos (≤ 75 m). Os autores concluíram que a utilização destas ferramentas permitiu identificar um grupo mais amplo e específico de pessoas com maior risco de hanseníase. Trabalhos subsequentes, subsidiados por SIG, fortaleceram a ideia de níveis diferentes de risco, de acordo com a distância espacial dos casos registrados (92, 93).

Experiências com a análise da distribuição espacial da hanseníase no município de Mossoró, no Rio Grande do Norte, permitiram o direcionamento de campanhas de busca ativa de casos em regiões específicas onde havia uma maior concentração de casos. Estas campanhas resultaram em um significativo aumento do número de casos novos diagnosticados precocemente, além de uma importante diminuição dos custos financeiros (24, 94, 95).

Apesar das evidências disponíveis, nenhuma destas novas tecnologias tem sido aplicada no Pará, Estado com uma das maiores cargas da doença no Brasil. Os poucos dados sobre a distribuição espacial dos casos no Pará estão agregados por municípios ou regiões (32, 96, 97) e não permitem a localização de áreas críticas dentro das cidades para o

direcionamento das ações de controle. A melhoria da precisão para identificação de áreas críticas nos municípios paraenses poderia melhorar o gerenciamento das políticas públicas de combate à hanseníase no Pará.

1.10. Justificativa

Este cenário de hiperendemicidade histórica no Pará - com precária taxa de avaliação de contatos intradomiciliares (34% no período de 2003 a 2007) (28), evidências da existência de focos ativos de transmissão não diagnosticados, além de uma baixa cobertura do programa saúde da família no Estado (42%) (98) - indica a necessidade de implementar esforços extras e desenvolver novas tecnologias e estratégias de controle da hanseníase no Pará.

1.11. Objetivos

1.11.1. Objetivo geral

Desenvolver um método integrando a epidemiologia espacial e sorológica como ferramenta de combate à hanseníase no Estado do Pará.

1.11.2. Objetivos específicos

a. Identificar a soroprevalência de anticorpos anti-PGL-I em pessoas afetadas pela hanseníase, em seus contatos intradomiciliares e entre estudantes de escolas públicas do ensino fundamental e médio em municípios de diferentes regiões do Estado do Pará, além de determinar a prevalência previamente não diagnosticada da doença entre os contatos e os estudantes (Capítulos 2 e 3).

b. Mapear os casos registrados de hanseníase e analisar o padrão espacial da distribuição da doença em um município hiperendêmico do Pará, correlacionando a ocorrência de infecção subclínica e de novos casos da doença com a distribuição espacial dos casos notificados (Capítulo 4).

c. Descrever e avaliar uma estratégia para aumentar a detecção precoce de casos novos baseada na epidemiologia espacial e sorológica da hanseníase em um município hiperendêmico do Pará (Capítulo 5).

1.12. Desenho metodológico

Este estudo está em conformidade com a Declaração de Helsinki e foi aprovado pelo Comitê de Ética em Pesquisa do Instituto de Ciências da Saúde da Universidade Federal do Pará (protocolo número 197/07 CEP-ICS/UFPA) (anexo 1).

Inicialmente, foi realizado um estudo transversal por meio de visitas domiciliares a famílias de pessoas afetadas pela hanseníase, diagnosticadas nos últimos cinco a seis anos, em oito municípios localizados em diferentes regiões do Estado (Altamira, Breves, Castanhal, Marituba, Oriximiná, Paragominas, Parauapebas e Redenção).

Uma equipe de pesquisadores com experiência no manejo da hanseníase, composta por médicos dermatologistas, enfermeiros, fisioterapeutas e técnicos de laboratório, realizou exame clínico dermatoneurológico e entrevista socioeconômica padronizada (apêndices 1, 2 e 3) em 1.945 contatos intradomiciliares de 531 casos notificados e coletou amostra de sangue para pesquisa sorológica de anticorpos IgM anti-PGL-I. Além disso, 1.592 estudantes de 37 escolas públicas do ensino fundamental e médio, com idade entre 6 e 20 anos, também foram selecionados aleatoriamente para serem submetidos à mesma avaliação. As residências dos casos notificados, bem como a dos estudantes incluídos no estudo foram mapeadas para a análise da distribuição espacial da hanseníase e da infecção subclínica pelo *M. leprae*.

Dois anos mais tarde, a equipe de pesquisadores retornou a dois municípios (Oriximiná e Castanhal) para reavaliar os indivíduos incluídos no estudo e comparar o desfecho clínico de acordo com a informação sorológica prévia (coorte). Nesta segunda etapa do estudo, também foram selecionadas duas novas escolas públicas localizadas em áreas de alto risco de hanseníase, determinadas pela análise da distribuição espacial da doença em um dos municípios (Castanhal), para avaliação da importância da informação geográfica na detecção de casos novos da doença.

Os detalhes da metodologia utilizada estão descritos nos capítulos subsequentes (2 a 5), compostos pelos artigos completos resultantes deste estudo.

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CAPÍTULO 2

ANTI-PGL-I SEROEPIDEMIOLOGY IN LEPROSY CASES, HOUSEHOLD CONTACTS AND SCHOOL CHILDREN FROM A HYPERENDEMIC MUNICIPALITY OF THE BRAZILIAN AMAZON

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Summary

Objective: We investigated the prevalence of antibodies against PGL-I in people affected by leprosy (PAL) who were diagnosed and treated between 2004 and 2010, their household contacts (HC) and school children (SC) from a hyperendemic municipality in the Brazilian Amazon and determined the prevalence of previously undiagnosed leprosy (PPUL) among both the HC and SC.

Design: We conducted a cross-sectional study involving 87 PAL, 302 HC and 188 SC. The subjects were clinically assessed, and their levels of anti-PGL-I antibodies were determined by ELISA. The subjects were also interviewed to determine their demographic and socioeconomic characteristics.

Results: For PAL, a mean of 44 (SD=21.8) months had passed since their initial diagnosis, and 34 (39%) of them remained seropositive. The level of anti-PGL-I antibodies was significantly higher in multibacillary (MB) than in paucibacillary (PB) cases ($p<0.05$). Thirty-nine percent of HC were positive for anti-PGL-I, and we detected 8 (2.6%) new cases among these individuals. One hundred and twenty-five SC (66.5%) were seropositive, and we detected 9 (4.8%) new cases of leprosy (8 PB and 1 MB) in this group. When we visited the homes of SC affected by leprosy, 31 contacts were clinically examined, and three (10%) new cases were detected (one PB and two MB). The mean age of students with leprosy was 14.1 years (SD = 2.5; min = 10, max = 18).

Conclusion: The seroepidemiology of anti-PGL-I and the PPUL among both HC and SC suggests that there are many active foci of infection and that *Mycobacterium leprae* is circulating among this population.

Introduction

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, is under control in developed countries; however, 228,474 new cases were detected worldwide in 2010, mostly in developing countries. The registered prevalence at the first quarter of 2011 was 192,246 cases. Brazil, with 34,894 new cases detected in 2010, has the highest prevalence rate of leprosy in the world (1.56/10,000 people), registered at the end of first quarter 2011, and has yet to reduce its rate below the threshold of 1/10,000 people, the level at which leprosy would be considered eliminated as a public health problem.[1] The geographic distribution of leprosy is heterogeneous in Brazil. The more developed states in the South, such as Rio Grande do Sul, have already achieved the elimination target, whereas there are clusters of high endemicity in the North, Central-West and Northeast, suggesting a high concentration of leprosy cases in parts of Brazil. Most of the areas with clusters are in the Brazilian Amazon, which has been recognised as a highly endemic leprosy area.[2]

Case detection among children under 15 years old is correlated with recent disease and active foci of transmission in the community, reflecting the efficiency of local control programmes. The state of Pará in the Amazon region is hyperendemic in this age group, with an annual case detection rate of 20.4/100,000 people in 2008, much higher than the Brazilian average of 5.8/100,000 people,[3] indicating that *M. leprae* is circulating among this population of children.

Household contacts (HC) of untreated multibacillary (MB) leprosy patients are considered the main group at risk of contracting the disease. Early case detection and multidrug therapy (MDT) administered regularly and completely are the key components of a leprosy elimination programme.[4] The diagnosis of leprosy is based mainly on clinical examination, and there is no laboratory test that detects all forms of leprosy. Since phenolic glycolipid-I (PGL-I), a species-specific antigen from the *M. leprae* cell wall, was isolated and characterised,[5] various studies have noted the potential use of serology to detect antibodies against PGL-I to classify patients for treatment purposes, case monitoring, identifying the risk of relapse, and in the selection of HC at a higher risk of contracting the disease.[6] A positive test for anti-PGL-I is associated with an 8.6-fold higher leprosy risk in HC and a 4.4-fold higher risk in non-contacts.[7] Therefore, screening tests to determine the seroepidemiology in hyperendemic areas may be useful to identify subclinical infection among HC and children in the general population, although there are no conclusive data confirming this approach.

The objectives of this study were to: (1) detect the prevalence of IgM antibodies against PGL-I among people affected by leprosy (PAL), their HC and school children (SC)

from a hyperendemic municipality in the Brazilian Amazon; (2) detect the prevalence of new leprosy cases among this population, and (3) identify clinical, demographic and socioeconomic characteristics that contribute to seropositivity and disease.

Methods

This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethics Committee from the Federal University of Pará (protocol number 197/07 CEP-ICS/UFPA).

Study design, setting and population

We conducted a cross-sectional study in the municipality of Castanhal, state of Pará, Brazil, in June 2010. Castanhal is 68 km from Belém, the state capital, and its total population was 173,096 inhabitants in 2010. There were 82 public elementary and high schools with a total of 39,331 students in 2010.[8, 9] According to the municipal secretary of health, there were 633 newly detected leprosy cases from 2004 to February 2010. The annual case-detection rate among the general population was 48.2/100,000 inhabitants in 2009; among children under 15 years old, the case-detection rate was 10.3/100,000, classifying the municipality as hyperendemic according to the parameters designated by the Brazilian Ministry of Health.

Participants

Leprosy is a compulsory notifiable disease in Brazil; thus, all patients have their clinical data and addresses registered in the national notifiable diseases information system (SINAN). We identified patients' addresses in cooperation with the municipal secretary of health. A random sample of 100 subjects from 11 urban neighbourhoods and 1 rural village, identified as leprosy cases from 2004 to February 2010, were electronically selected according to a sequence generated by BioEstat 5.0 software (Sociedade Civil Mamirauá, Amazonas, Brazil). These individuals were visited at their homes by a team of health care professionals with experience in treating leprosy patients, including a dermatologist, a nurse, a physical therapist, a lab technician and a local community health agent. The subjects and their HC were clinically assessed for signs and symptoms of leprosy, and a sample of peripheral blood from each person was collected to identify the prevalence of IgM antibodies against PGL-I. They were also interviewed about their demographic and socioeconomic characteristics using a standard questionnaire.

Eligibility criteria were as follows: (1) residing at the same address as when they were diagnosed, (2) being home at the time of health care team visit and (3) providing written, informed consent to participate in the study. When a participant was younger than 18 years old, consent was obtained from parents or another responsible adult in the family. There were no restrictions on gender, skin colour, age group, or comorbidity. When at least one eligibility criterion was not met, another patient home in the same neighbourhood was selected from a randomly generated reserve list.

In addition to evaluating cases and contacts, we also randomly selected 200 students between six and 18 years old from four public schools (two elementary and two high schools) located in four different peripheral neighbourhoods. Teachers, selected students and their parents received information about general aspects of leprosy, followed by visits from the team of health care professionals, who clinically evaluated the students and collected blood samples from those who consented to participate in the study. When a new case was detected among the students, we went to the student's home to evaluate household contacts.

The diagnosis of a new case among PAL, HC or SC was done by the finding of a skin lesion with loss of sensation. After that, the case was classified as indeterminate leprosy when there was only a hypopigmented macule, with no detection of nerve involvement (PB cases), or as one of the clinical forms defined by the Ridley-Jopling classification (TT as PB cases, while BT, BB, BL or LL were classified as MB cases).

Laboratory procedures

Peripheral blood samples (4ml) were collected aseptically in EDTA tubes by an experienced technician; plasma was separated by centrifugation (2000rpm/5min) and then stored at -80°C until use. For analysis, samples were defrosted and immediately tested at the Dermato-Immunology Laboratory in Marituba, Pará, Brazil. Seropositivity was determined by enzyme-linked immunosorbent assay (ELISA).

The following ELISA protocol was used: native PGL-I (batch 9/10/10 pool 16/20), generously provided by Dr. John Spencer of Colorado State University (USA), was coated onto 96-well, flat polystyrene microtitre plates (Kartell S.P.A., Novigilio, Milan, Italy) using 0.5 µg/well in 50 µl of ethanol, and plates were stored overnight in a fume hood at room temperature (RT) to evaporate the ethanol from the wells. The plates were blocked for one hour using 200 µl/well of PBS, pH 7.2, containing 3% bovine serum albumin (BSA, Sigma-Aldrich A7906, St. Louis, MO, USA) (blocking solution), then washed twice with the same solution. Two microlitres of each plasma sample was diluted 1:200 in 400 µl blocking

solution, and 100 µl of this solution was added to the wells in duplicate and to 1 blank control well (not coated with PGL-I) for each subject and incubated for 2 hours at RT. After incubation with plasma, the wells were washed five times with 0.3% BSA-PBS (washing solution) using an automatic plate washing machine (Ultrawash plus, Dynex Technologies, Chantilly, VA, USA) and then one more time with blocking solution. Then, 100 µl/well of secondary anti-human IgM antibody (µ-chain specific) conjugated with peroxidase (Sigma-Aldrich A0420, St. Louis, MO, USA) at a 1:10,000 dilution in blocking solution was added and incubated for 2 hours at RT. After incubation with the conjugate, the wells were washed five times with washing solution, one time with blocking solution, and then two more times with PBS only. After washing, 100 µl of o-Phenylenediamine dihydrochloride substrate (SIGMA*FAST*™ OPD, Sigma-Aldrich P9187, St. Louis, MO, USA) was added. After 30 minutes, the reaction was stopped with 50 µl of sulphuric acid solution (4N), and after 10 minutes, the absorbance was read at 490nm using an MRX Revelation 4.25 microplate reader (Dynex Technologies, Chantilly, VA, USA). The cut-off for positive results was arbitrarily established at an optical density (OD) of 0.295, based on the average plus 3 times the standard deviation of the test results from 14 healthy subjects from the same hyperendemic area. The final OD value of each plasma sample was determined by subtracting the OD background of the blank control well from the mean of the duplicates from each respective subject. To control for each plate, a positive plasma sample from a high bacilloscopic index (BI) lepromatous leprosy patient and a negative sample from an US-born healthy person with no known exposure to leprosy who lives in Colorado (USA) were included in duplicate on each plate. An external validation was performed to assure the quality of the laboratory procedures and reagents used.

Data analysis

The collected data were submitted to descriptive analysis and methods of statistical inference using BioEstat 5.0 software. Statistical significance was assessed using a significance level of 0.05 (two-tailed). Student's *t*-test or Mann-Whitney *U* test was used to assess quantitative data from independent samples. A Chi-squared or Fisher's exact test was used to compare proportions between different groups when appropriate.

Results

Of the 100 randomly selected PAL, 87 were visited at their homes by the team of health care professionals and were included in the present study. During these visits, we were

also able to evaluate 302 HC. Thirteen patients were excluded because we were unable to find their residences; they had moved away, had died or did not consent to participate. Of the 200 students selected for the examination of SC, 188 were evaluated. Twelve students were excluded because their parents did not attend the meeting with the researchers to provide written consent for their children's participation.

The mean age of PAL included in this study was 39.8 years old (SD = 17.6; min = 12, max = 91). At the time of clinical examination and blood collection, a mean of 44 (SD = 21.8; min = 5, max = 84) months has passed since the initial diagnosis of these individuals; 34 (39%) of them were positive for anti-PGL-I; 81 (93%) lived in an urban area; 32 (37%) were illiterate or functionally illiterate; 31 (36%) had completed elementary school and 24 (28%) had completed high school; 21 (24%) indicated that they had experienced starvation at least once, as defined by a full day without meals, because of the absence of resources to buy food [IBGE]; 65 (75%) had family incomes of up to twice the Brazilian minimum wage (approximately 695 US dollars), including 56 (64%) that received some kind of governmental financial assistance, such as family allowance and retirement benefits; and 35 (40%) lived in a house with more than 2 people per bedroom.

According to the degree of physical disability at the time of diagnosis, discharging by cure and the day of evaluation in this study, 41 people (47%) started and finished treatment with no disability, 26 (30%) were diagnosed with some degree of disability and improved during or after the MDT, and 13 (15%) patients experienced diminished functional capacity after discharge by cure. Table 1 shows the seroprevalence in PAL according to sex, age group, WHO classification, the number of BCG scars and the duration since diagnosis.

The levels of anti-PGL-I antibodies were significantly higher in MB than in PB cases (Figure 1), but there was no difference between those who became more physically impaired and those who remained well or experienced improvements in their levels of disability with MDT (p = 0.160; 95% CI = -0.63 to 0.10). The mean elapsed time since diagnosis among seronegative and seropositive PAL was 46.2 and 40.7 months, respectively, and there was no significant difference between these groups (p = 0.272; 95% CI = -15.44 to 4.42).

The mean age of HC was 28.8 years old (SD = 17.6; min = 3, max = 81), and 126 (42%) were male. Table 2 presents the seroprevalence among contacts according to sex, age group, index case classification and the number of BCG scars.

Of the 302 HC evaluated, 118 (39%) were positive for anti-PGL-I; 6 (2%) were previously affected by leprosy and completed the MDT. Furthermore, we detected eight new

cases among these individuals, corresponding to 2.6% of all of the HC clinically examined (four PB and four MB).

Table 1. The seroprevalence in leprosy cases[*] according to sex, age group, WHO classification, the number of BCG scars and the elapsed time since diagnosis.

		Seropositive		Seronegative			
Category	**Subcategory**	n	%	N	%	OR (95% CI)	*p*-value[#]
Sex	Male	14	36.8	24	63.2	0.84 (0.35–2.02)	0.706
	Female	20	40.8	29	59.2	1.18 (0.49–2.82)	
Age group	< 15 years old	1	20	4	80	0.37 (0.04–3.47)	0.347
	≥ 15 years old	33	40.2	49	59.8	2.69 (0.28–25.1)	
Classification	Paucibacillary	8	30.8	18	69.2	0.59 (0.22–1.58)	0.299
	Multibacillary	26	42.6	35	57.4	1.67 (0.63–4.43)	
BCG scar[1]	None	16	42.1	22	57.9	1.22 (0.50–2.99)	0.115
	One	14	34.1	27	65.9	0.63 (0.25–1.55)	
	Two	2	100	—	—	!	
Elapsed time since diagnosis	≤ 36 months	18	47.4	20	52.6	1.85 (0.77–4.44)	0.162
	> 36 months	16	32.7	33	67.3	0.54 (0.22–1.29)	

[*]Cases detected from 2004 to February 2010, evaluated cross-sectionally in June 2010.
[#]Chi-squared test.
[1]Six subjects had uncertain BCG scars and were not included in this analysis.
[!]The odds ratio could not be calculated because of the null frequency of the matrix.
Note: WHO = World Health Organisation. BCG = Bacillus Calmette-Guérin. OR = odds ratio. CI = confidence interval.

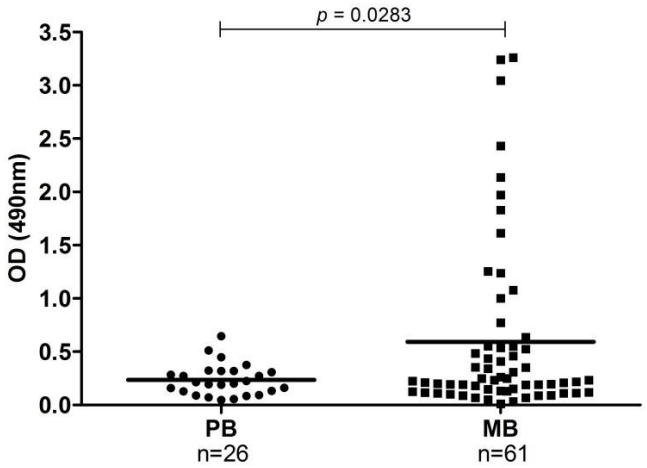

Figure 1. The levels of anti-PGL-I in people affected by leprosy according to WHO classification.
Note: WHO = World Health Organisation. PB = Paucibacillary. MB = Multibacillary. OD = Optical Density. Statistical differences between groups were calculated using Student's *t*-test (2-tailed), 95%CI = -0.67 to -0.03.

Table 2. The seroprevalence among household contacts according to sex, age group, index case classification, the number of BCG scars and the number of clinically detected new cases.

		Seropositive		Seronegative			
Category	**Subcategory**	n	%	N	%	OR (95% CI)	*p*-value*
Sex	Male	46	36.5	80	63.5	0.83 (0.52–1.33)	0.439
	Female	72	40.9	104	59.1	1.20 (0.75–1.93)	
Age group	< 15 years old	30	44.1	38	55.9	1.31 (0.76–2.27)	0.332
	≥ 15 years old	88	37.6	146	62.4	0.76 (0.44–1.31)	
Index case classification	Paucibacillary	34	35.8	61	64.2	0.81 (0.49–1.35)	0.428
	Multibacillary	84	40.6	123	59.4	1.22 (0.74–2.03)	
BCG scar	None	12	25.5	35	74.5	0.48 (0.23–0.97)	0.109
	One	76	40.9	110	59.1	1.22 (0.75–1.96)	
	Two	30	43.5	39	56.5	1.26 (0.73–2.18)	
New cases detected		4	50	4	50		

Note: BCG = Bacillus Calmette-Guérin. OR = odds ratio. CI = confidence interval. *Chi-squared test.

Among SC, 125 (66.5%) tested positive for anti-PGL-I. We detected nine new cases in students (eight PB and one MB, 88.9% anti-PGL-I positive) and visited seven of them at their homes (two addresses were not found). During these visits, 31 HC of the newly diagnosed students were also clinically examined, and three (10%) additional new cases were detected (one PB and two MB). Only 18 (58%) of the students' HC were BCG vaccinated, and nine (29%) were positive for anti-PGL-I, including 2 of the 3 new cases. The mean age of students with leprosy was 14.1 years old (SD = 2.5; min = 10, max = 18), and none of them had disabilities at the time of diagnosis. Table 3 presents the demographic characteristics, BCG vaccination history, rate of new case detection, and seroprevalence in students per visited school. Table 4 presents the OD values in PAL, HC and SC.

There was no correlation between SC age and seroprevalence (Pearson's correlation coefficient, $p = 0.142$; 95% CI = -0.04 to 0.25), but when we grouped HC and SC, there was a significant difference between the proportion of BCG-vaccinated individuals who were seropositive compared to unvaccinated subjects (Figure 2).

Table 3. The demographic characteristics, BCG vaccination, rate of new case detection, and seroprevalence of students per school.

	School				Total
	A	**B**	**C**	**D**	**4**
Enrolled students in 2010	660	504	755	1,477	**3,396**
Evaluated students	49	47	42	50	**188**
Age, mean (SD)	8.8 (1.3)	11.1 (1.8)	16.5 (1.9)	16 (1.5)	**13 (3.6)**
Sex (M/F)	20/29	29/18	17/25	9/41	**75/113**
BCG vaccination (%)	45 (92)	46 (98)	41 (98)	47 (94)	**179 (95)**
New cases detected (%)	0 (0.0)	3 (6.4)	3 (7.1)	3 (6.0)	**9 (4.8)**
Seroprevalence (%)	31 (63.3)	27 (57.4)	31 (73.8)	36 (72)	**125 (66.5)**
OD of seropositives, median (IQR)	0.453 (0.284)	0.446 (0.335)	0.593 (0.211)	0.581 (0.385)	**0.543 (0.287)**
OD of seronegatives, median (IQR)	0.178 (0.035)	0.208 (0.078)	0.185 (0.107)	0.230 (0.095)	**0.191 (0.083)**

Note: IQR = interquartile range. M/F = male/female. BCG = Bacillus Calmette-Guérin. OD = optical density read at 490nm. There were no statistically significant differences in the proportions of BCG vaccination, new case detections or seroprevalence among the different schools (Chi-squared test, $p>0.05$).

Table 4. The optical density (OD) values in leprosy cases,[*] household contacts and school children.

Category	**Median OD (IQR)**	**Subcategory[a]**	**Median OD (IQR)**	***p*-value[b]**
People affected by leprosy[*]	0.224 (0.324)	Seropositive	0.543 (0.866)	< 0.0001
		Seronegative	0.152 (0.116)	
Household contact	0.216 (0.290)	Seropositive	0.467 (0.302)	< 0.0001
		Seronegative	0.128 (0.124)	
School children	0.377 (0.365)	Seropositive	0.543 (0.287)	< 0.0001
		Seronegative	0.191 (0.083)	

Note: OD = optical density read at 490nm. IQR = interquartile range.
[*]Cases detected from 2004 to February 2010, evaluated cross-sectionally in June 2010.
[a]The cut-off for positivity = 0.295 OD. [b]Mann-Whitney *U* test (two-tailed).

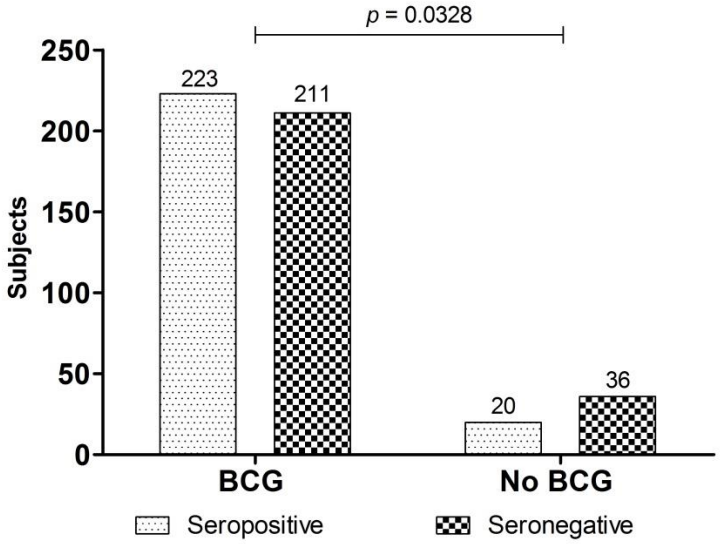

Figure 2. The seroprevalence of anti-PGL-I according to BCG vaccination status.
Note: BCG = Bacillus Calmette-Guérin. This sample represents all household contacts and school children evaluated. Statistical differences between groups were calculated using Fisher's exact test (2-tailed); 95% CI = 1.06 to 3.39.

Discussion

The former team leader of the WHO's Global Leprosy Programme, Vijaykumar Pannikar, has indicated that Brazil's control efforts focus almost entirely on urban areas, to the detriment of poor northeastern and Amazon regions, where leprosy is particularly acute.[10] Our study demonstrates that *M. leprae* infection is widely spread in this sample of the Amazon population, especially among school children. Official data indicate that the State of Pará has the highest known number of new cases detected among children less than 15 years old in Brazil (8,938 cases, from 1994 to 2007).[11] Furthermore, there is evidence that in highly endemic areas, the prevalence of previously undiagnosed leprosy in the general population is six times higher than the registered prevalence.[12]

According to the Brazilian Leprosy Control Programme (PNCH), all HC of newly detected cases should be examined, but the average proportion of examinations, which has been considered precarious since 2002, was only 41% in Pará.[3] Case detection is based mainly on spontaneous demand at health facilities. When we visited the houses of PAL and evaluated their HC, we detected a number of new cases that would correspond to a prevalence rate of 265/10,000 people in this specific group. These clinical findings, along with the high proportion of seropositivity, confirm that the HC of newly detected cases must be examined

and also indicate that HC should be examined not only at the time of index case detection but also periodically, perhaps annually, during the average period of disease incubation.

In June 2010, we examined 13.7% of all PAL diagnosed from 2004 to February 2010 in Castanhal in a cross-sectional study. It is expected a reduction in the serum levels of antibodies against PGL-I after the start of MDT. This decline is typically 25 to 50% per year, but in some patients, the decline is slower, and seropositivity can remain for years after an official cure.[13-15] The high rate of seroprevalence observed in this sample of former patients could be the result of (1) dead or inactive bacteria that are still present in PAL; (2) constant contact with an untreated source of infection; or (3) a first indicator of a relapse, given that seropositivity is a risk factor in the future development of relapses, especially in those groups of patients who have received a shorter than usual course of treatment.[16, 17] However, we did not evaluate the antibody levels at the beginning of treatment, thus it is possible that those values were higher than that at the moment of examination, meaning that the anti-PGL-I titers is simply gradually declining after MDT.

Despite little change in incidence rates of leprosy, one study shows that the disease is more common in people with insufficient education in lower social classes than in other population groups, and that this reality is more stark and systematic in the Amazon region of Brazil than in other areas of this country.[18] This characteristic was observed in our study. Notably, Pará has one of the highest population densities per domicile in Brazil (4.1 people per residence),[19] and we found almost half of PAL living in a home with more than two people per bedroom, with up to 15 people living in a single impoverished house. This fact probably contributes to the dissemination of communicable diseases like leprosy; it has been shown that people living in households with more than seven members have a 3.1-fold higher risk of contracting disease than people living in households of 1-4 members.[20]

In addition, Feenstra and colleagues[21] stated that a recent period of food shortage was significantly associated with clinical manifestation of leprosy. We detected that the proportion of PAL who experienced starvation (24%) was much higher than that of the general population in the North of Brazil (9%), and almost five fold higher than the national average (5%).[22] Subnutrition could impair the effectiveness of immunological system,[23] and should be target as part of the leprosy control strategy.

Some PAL experienced a worsening in their levels of disability after finishing MDT. It has been observed in other studies that approximately 5% of PB and 20% of MB patients develop sensitivity losses after their official cure.[24] We failed to find any correlation between

the degradation of functional capacity and the levels of anti-PGL-I, although both symptoms have been directly correlated with high bacilloscopic index (BI) and MB leprosy.[25-28]

Serology is useful for the identification of persons with an increased risk of developing leprosy among high-risk groups such as contacts.[16,20,29] Studies of the seroprevalence among HC have reported seropositivity of approximately 1.9% to 18.4%,[6] but our results are significantly higher than these published data. Such variance may be attributed to differences in the level of regional endemicity.[30] The ELISA protocols are also different among studies, and disparities such as sera dilution, the type of antigen and the cut-off for positive results could partially explain the variability of the results. Of 8 new cases detected among HC, four were clinically classified as PB, three of them seronegative for anti-PGL-I; and 4 were clinically classified as MB, three of them seropositive for anti-PGL-I. These findings are in accordance with previously published data[16].

The high seroprevalence and, particularly, the elevated prevalence of previously undiagnosed new cases among SC were the most surprising results of this study. These data clearly suggest the presence of several active foci of infection and that *M. leprae* is widely circulating among this population. If this finding is reproducible for the entire population of SC from the public schools of Castanhal, it is possible that approximately 1,884 undiagnosed cases exist among SC at this time (a rate of 479/10,000 students).

While 66.5% of the SC were anti-PGL-I positive, eight (88,9%) of nine SC newly detected as leprosy cases were seropositives, indicating a possible role for anti-PGL-I in functioning as a tool for detection of SC with a higher risk of developing leprosy. However, the present sample is not sufficient to evaluate this hypothesis, and new studies are necessary to elucidate this.

Five (55%) of the newly diagnosed students were younger than 15 years old, and two of their HC evaluated and also diagnosed with leprosy during visits to their homes were also children under 15 years old. Single skin-lesion paucibacillary leprosy was the most common clinical form found in these cases. Such clinical presentation is often not detected by less experienced clinicians, nor is it noticed by the patient. The total number of new cases detected among children during this cross-sectional study (nine, including HC + SC + contacts of SC) was almost two-fold higher than that detected during the entire year of 2009 in Castanhal (five, according to notifications registered at SINAN up to May 2011).

Another unexpected and important result was the significant association between seropositivity and BCG vaccination status. We assume that people who were vaccinated have a higher tendency to develop immunological surveillance against *Mycobacterium spp.*

infections. Thus, once infected, they may be more likely to produce antibodies. Other studies have found a similar association, but without statistical significance.[31,32] One group of researchers hypothesised that the high reactivity toward *M. leprae* lipoarabinomannan (LepLAM), a cell wall antigen, in healthy volunteers who did not have any known exposure to either tuberculosis (TB) or leprosy in a region where leprosy and TB are endemic could be the result of increased exposure to pathogenic or avirulent environmental mycobacteria (for example, in the soil and water) or to BCG vaccination.[27]

It has been shown that infants produce and progressively increases detectable serum levels of anti-PPD IgM after a single BCG dose;[33] that BCG coupled to homologous anti-BCG serum IgM lead to more rapid necrosis and resolution of experimental granulomas in rats[34] and that melanoma GM2 protein coupled to BCG induces more IgM antibodies than GM2 or BCG alone[35] The high levels of anti-PGL-I IgM found in SC may be explained by the co-occurrence of early *M. leprae* exposure and BCG, inducing and maintaining high IgM levels, which may decrease after effective cellular immune response, as shall occur in the majority of the population.

We also analysed the seroepidemiological results using a receiver operating characteristic (ROC) curve. This analysis indicated a cut-off point of 0.411 (OD), with a sensitivity of 88.9% and a specificity of 56.4%. Even considering these data with a higher cut-off for positivity, the seroprevalence among SC remains extremely elevated (45.2%). These findings are substantially higher than those observed in other similar studies of SC.[30,36]

We presume that people from hyperendemic areas are exposed to infection starting in early childhood and develop some degree of immunological response. Godal and Negassi[37] reported that approximately 50% of subjects with household or occupational contact with leprosy for at least a year have immunological evidence of exposure to *M. leprae*, indicating that the subclinical infection rate is much higher than the prevalence of the disease.

Our data indicate that children may be infected very early, produce IgM antibodies for months or years, and then for genetic, immunological or less probable *M. leprae* strain reasons, shift for a good Th1 cellular immune response, eliminating the bacteria. Some of them may not develop a good cellular immune response or may not develop at all, and will present the different clinical forms of leprosy.

It seems that the majority of people infected by *M. leprae* will never present clinical signs and symptoms of leprosy, harbouring a subclinical infection for some time, but that a considerable number of people will be affected by the disease. The main challenge is to discover who will become ill and what can be done to prevent it.

Chemoprophylaxis has been associated with reduced leprosy incidence in the first years after implementation.[38-42] The use of single-dose rifampicin given to contacts of newly diagnosed leprosy patients is a cost-effective intervention strategy, but further research should be conducted to evaluate the impact of this treatment strategy on endemicity of the Brazilian Amazon region.

The seroepidemiology of anti-PGL-I and the PPUL among both HC and SC suggest that there are many active foci of infection and that *M. leprae* is circulating among this population. Therefore, it is necessary to identify and properly handle the hidden prevalence of leprosy in the Brazilian hyperendemic municipalities, otherwise, the targets of the leprosy elimination programme will not be achieved in the coming decades for these areas of the world.

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CAPÍTULO 3

HIGH RATES OF UNDIAGNOSED LEPROSY AND SUBCLINICAL INFECTION AMONGST SCHOOL CHILDREN IN THE AMAZON REGION

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Summary

Leprosy in children is correlated with community-level factors, including the recent presence of disease and active foci of transmission in the community. We performed clinical and serological examinations of 1,592 randomly selected school children (SC) in a cross-sectional study of eight hyperendemic municipalities in the Brazilian Amazon Region. Sixty-three (4%) SC, with a mean age of 13.3 years (standard deviation = 2.6), were diagnosed with leprosy and 777 (48.8%) were seropositive for anti-phenolic glycolipid-I (PGL-I). Additionally, we evaluated 256 house-hold contacts (HHCs) of the students diagnosed with leprosy; 24 (9.4%) HHC were also diagnosed with leprosy and 107 (41.8%) were seropositive. The seroprevalence of anti-PGL-I was significantly higher amongst girls, students from urban areas and students from public schools ($p < 0.0001$). Forty-five (71.4%) new cases detected amongst SC were classified as paucibacillary and 59 (93.6%) patients did not demonstrate any degree of physical disability at diagnosis. The results of this study suggest that there is a high rate of undiagnosed leprosy and subclinical infection amongst children in the Amazon Region. The advantages of school surveys in hyperendemic areas include identifying leprosy patients at an early stage when they show no physical disabilities, preventing the spread of the infection in the community and breaking the chain of transmission.

Key words: leprosy - epidemiology - anti-PGL-I - subclinical infection - school children

Introduction

Leprosy in children younger than 15 years old is correlated with recent disease and active foci of transmission in the community, reflecting the efficiency of local control programmes. In the state of Pará (PA), in the Brazilian Amazon Region, leprosy is hyperendemic in this age group. This state had an annual case detection rate of 20.4/100,000 people in 2008, which is much higher than the Brazilian average of 5.8/100,000 people (MS/SVS 2009), indicating that *Mycobacterium leprae* is circulating amongst the children in PA. Furthermore, in highly endemic areas, the prevalence of previously undiagnosed leprosy in the general population is six times higher than the registered prevalence (Moet *et al.* 2008).

In Brazil, the primary health service is responsible for diagnosing leprosy, finding active cases of leprosy, tracing the patients' contacts, treating leprosy and preventing disability in the people affected by leprosy, but only 42% of the total population of PA is covered by these services (Department of Health Care/Department of Primary Care 2012 - dab.saude.gov.br/historico_cobertura_sf.php). This scenario suggests that there may be many patients with undiagnosed leprosy in PA who are perpetuating the transmission of the bacillus. Additionally, because of the long incubation period of *M. leprae*, more leprosy patients (LPs) are expected to emerge in the coming decades.

The diagnosis of leprosy is based primarily on a clinical examination and there is no laboratory test that detects all forms of leprosy. Because of the isolation and characterisation of phenolic glycolipid-I (PGL-I) (Hunter & Brennan 1981), a species-specific antigen from the *M. leprae* cell wall, various studies have demonstrated that serology could potentially be used to detect antibodies against PGL-I to classify patients for treatment purposes, monitor cases, identify the risk of relapse and identify the household contacts (HHCs) of LPs who are at a higher risk of contracting the disease than the general public (Moura *et al.* 2008). A positive test for anti-PGL-I is associated with an 8.6-fold higher risk of leprosy in HHCs and a 4.4-fold higher risk in non-contacts (Brasil et al. 2003).

Some studies have shown that subclinical infections with *M. leprae* are much more common than overt disease in endemic communities (Godal & Negassi 1973, Abe *et al.* 1990, Barreto *et al.* 2011) and that anti-PGL-I seropositivity is a marker of subclinical infection (Baumgart *et al.* 1993, Lobato *et al.* 2011). Van Beers *et al*. (1999) indicated that the seropositivity rates amongst school children (SC) may reflect leprosy incidence.

Seroprevalence may be an appropriate indicator of the magnitude of the burden of leprosy in a selected area.

Therefore, screening to determine the seroepidemiology of anti-PGL-I in hyperendemic areas may be useful in identifying subclinical infections amongst children in the general population. In addition, combined with clinical examinations of randomly selected subjects, screening tests could help to estimate the true burden of the disease in a specific region. Thus, the objectives of this study were to determine the prevalence of subclinical infection (defined in this study as seropositivity for anti-PGL-I IgM with no clinical signs or symptoms of leprosy) and the prevalence of undiagnosed leprosy amongst SC from selected municipalities in PA.

Subjects, Materials and Methods

Study design, setting and population - We conducted a cross-sectional study in eight inner counties of PA, from 2009-2011. Table I shows the demographics and epidemiological characteristics of the selected municipalities and Fig. 1 illustrates their geographic locations. The PA counties were selected based on their geographic position to sample all of the regions of the state and to accommodate PA's leprosy clusters 1 and 7, as identified by Penna *et al.* (2009).

Participants - A team of health care professionals with experience in leprosy, including dermatologists, nurses, physical therapists, researchers and a lab technician, travelled to the municipalities and visited 37 randomly selected public elementary and high schools (Fig. 2). At each school, two-four classes (approximately 60 subjects) of students aged six-20 years were randomly selected. The teachers, selected students and their parents received general information about leprosy and the main objectives of the study. The students who agreed to participate provided their written consent; for the participants younger than 18 years old, consent was obtained from the parents or another responsible adult within the family.

Next, the students were clinically evaluated by a dermatologist and a sample of peripheral blood was collected from each subject to determine the prevalence of IgM antibodies against PGL-I. When a new leprosy case was detected amongst the students, we travelled to their homes to evaluate the HHCs of the students diagnosed with leprosy. There were no restrictions on study participation based on gender, skin colour or comorbidities. To

compare the anti-PGL-I titration levels of students with well-established leprosy cases, we included 51 patients [41 multibacillary (MB) and 10 paucibacillary (PB)] diagnosed at the Dr Marcello Candia Reference Unit in Sanitary Dermatology (UREMC) in PA. Additionally, 45 healthy students, aged seven-17 years, from private schools in Belém, PA's capital, were sampled and evaluated for anti-PGL-I titration levels.

TABLE I
Characteristics of the selected municipalities

Municipality	Population (2010)[a]	New cases detected (2006-2010)[b] (n)	Annual new case detection rate per 100,000 people (2009)[b]	Children among new cases of leprosy (2006-2010)[b] n (%)	Endemicity level[c]
Altamira	99,075	611	108.3	56 (9.2)	Hyperendemic
Breves	92,860	233	42.0	34 (14.6)	Hyperendemic
Castanhal	173,149	380	48.2	35 (9.2)	Hyperendemic
Marituba	108,246	424	50.4	65 (15.3)	Hyperendemic
Oriximiná	62,794	68	18.7	5 (7.3)	Highly endemic
Paragominas	97,819	720	130.4	80 (11.1)	Hyperendemic
Parauapebas	153,908	1,397	142.0	143 (10.2)	Hyperendemic
Redenção	75,556	584	186.4	72 (12.3)	Hyperendemic
Total	863,407	4,491	55.7[d]	490 (10.9)	-

a: source: Brazilian Institute for Geography and Statistics (ibge.gov.br/estadosat/); *b*: calculated from The National Notifiable Diseases 2012 (portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idtxt=31200); *c*: according to the parameters designated by the Brazilian Ministry of Health; *d*: average detection rate of the state of Pará (The National Notifiable Diseases 2012 - portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idtxt=31200).

Diagnostic procedures - The diagnosis of a new leprosy case was based on the identification of a skin lesion with sensory loss. A case was classified as indeterminate leprosy if there was a hypopigmented macule, but no detection of nerve involvement, or the case was classified as one of the clinical forms defined by the Ridley and Jopling (1966) classification system [tuberculoid-tuberculoid (TT), borderline tuberculoid (BT), borderline-borderline, borderline lepromatous or lepromatous-lepromatous (LL)]. Cases of indeterminate and TT leprosy were classified as PB cases, while the other forms were classified as MB cases. Primary neural leprosy was diagnosed if nerve enlargement was detected, but no skin signs were present. When only one nerve was affected, the case was classified as PB; two or more enlarged nerves defined the case as MB.

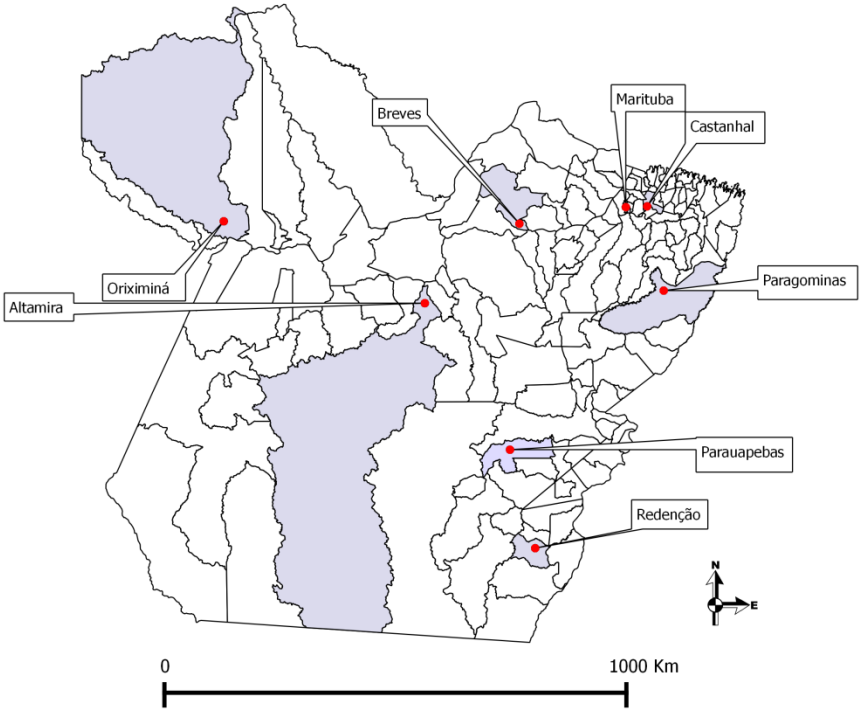

Fig. 1: Geographic locations of the selected municipalities in the state of Pará, in the Brazilian Amazon Region.

Laboratory procedures - Seropositivity was determined with an enzyme-linked immunosorbent assay (ELISA) using native PGL-I, which was generously provided by Dr John Spencer of Colorado State University (USA). The cut-off for positive results was arbitrarily established at an optical density (OD) of 0.295, based on the average plus three times the standard deviation of the test results from 14 healthy subjects from the same hyperendemic area (endemic control). A detailed description of the laboratory procedures was previously reported (Barreto *et al*. 2011).

Data analysis - Descriptive statistics were calculated and various statistical inference procedures were performed with BioEstat 5.0 software (Institute for Sustainable Development Mamirauá, Tefé, Amazonas, Brazil). Statistical significance was assessed using a significance level of 0.05 (two-tailed). The Student's t-test or the Mann-Whitney U test was used to assess the quantitative data from independent samples. A chi-square test or Fisher's exact test was used to compare proportions between different groups when appropriate and Pearson's coefficient was used to detect correlations between anti-PGL-I titres and the variables of interest.

Ethics - This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethical Committee at the Federal University of Pará (protocol 197/07 CEP-ICS/UFPA).

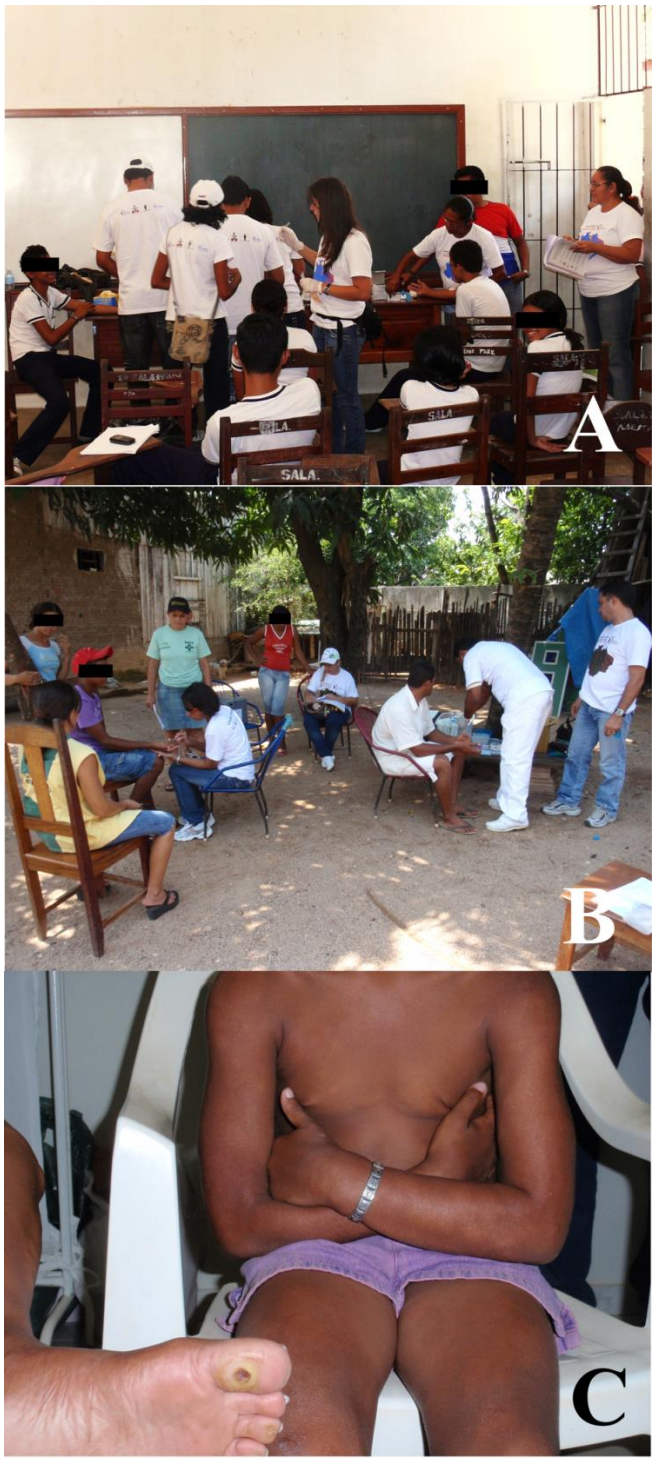

Fig. 2A, B: Sample pictures of team work on the school and on the field; **C**: One of the children diagnosed with borderline lepromatous leprosy with her father, detected after our visit to her house.

Results

A total of 1,592 students were examined (966 girls and 626 boys); the mean age was 12.3 [standard deviation (SD) = 3.2] years. Sixty-three (4%) of these students were clinically diagnosed with leprosy and 777 (48.8%) tested positive for anti-PGL-I IgM. We also examined 256 HHCs of the students diagnosed with leprosy at their homes (142 females and 114 males); the mean age was 25.7 (SD = 17.8) years. Twenty-four (9.4%) of the HHCs were also diagnosed with leprosy (Table II) and 107 (41.8%) tested positive for anti-PGL-I IgM. Table II shows the seroprevalence and the number of new cases detected amongst the students and HHCs in each municipality. Amongst the new cases detected, 51 (58.6%) were children younger than 15 years old. The distribution of cases by gender, age group, clinical classification, degree of physical disability, anti-PGL-I seropositivity and the presence of a BCG scar is shown in Table III.

TABLE II

Seroprevalence and new cases detected in each municipality

Municipality	Public schools[a] (enrolled students) n (%)	Selected schools (examined students) n (%)	Seroprevalence among students n (%)	New cases detected among students n (%)	HCSDL survey (n)	Seroprevalence among HCSDL n (%)	New cases detected among HCSDL n (%)
Altamira	115 (24.137)	6 (282)	81 (28.7)	10 (3.5)	30	9 (30)	1 (3.3)
Breves	325 (30.290)	5 (229)	150 (65.5)	14 (6.1)	81	30 (37)	12 (14.8)
Castanhal	82 (39.331)	4 (188)	125 (66.5)	9 (4.8)	31	9 (29)	3 (9.7)
Marituba	57 (24.978)	4 (199)	128 (64.3)	10 (5)	34	19 (55.9)	3 (8.8)
Oriximiná	89 (16.785)	6 (135)	57 (42.2)	6 (4.4)	25	14 (56)	2 (8)
Paragominas	93 (24.182)	4 (181)	84 (46.4)	10 (5.5)	44	22 (50)	3 (6.8)
Parauapebas	50 (33.300)	5 (146)	58 (39.7)	3 (2)	8	3 (37.5)	0 (0)
Redenção	37 (17.581)	3 (232)	94 (40.5)	1 (0.4)	3	1 (33.3)	0 (0)
Total	848 (210.584)	37 (1.592)	777 (48.8)	63 (4)	256	107 (41.8)	24 (9.4)

a: elementary and high schools (Brazilian Institute for Geography and Statistics - ibge.gov.br/estadosat/); HCSDL: household contact of student diagnosed with leprosy.

The levels of anti-PGL-I were similar in the MB patients (median OD = 0.369; IQR = 0.409) and the PB patients [median OD = 0.394; interquartile range (IQR) = 0.445] diagnosed during the active survey of the students and their HHCs (p = 0.752) and the proportions of seropositivity were also similar (MB = 60.6%, PB = 66.7%; p = 0.646). However, when we compared the MB and the PB patients diagnosed at the leprosy reference unit, significant differences were found in both the levels of anti-PGL-I (p = 0.007) and the proportions of seropositivity (MB = 95%, PB = 70%; p = 0.045). Figs 3, 4 illustrate the levels of anti-PGL-I in the different groups and Table IV shows the median OD values from the ELISA.

TABLE III

Epidemiologic characteristics of the new cases detected

Category	Students survey n(%)	HCSDL survey n (%)	Total n(%)
Gender			
Male	26 (41.3)	21 (50)	38 (43.7)
Female	37 (58.7)	12 (50)	49 (56.3)
Age group			
< 15 years old	42 (66.7)	9 (37.5)	51 (58.6)
≥ 15 years old	21 (33.3)	15 (62.5)	36 (41.4)
Classification			
Paucibacillary	45 (71.4)	9 (37.5)	54 (62.1)
Multibacillary	18 (28.6)	15 (62.5)	33 (37.9)
Degree of disability			
0	59 (93.6)	20 (83.4)	79 (90.8)
1	4 (6.4)	2 (8.3)	6 (6.9)
2	0 (0)	2 (8.3)	2 (2.3)
ELISA anti-PGL-I			
Seropositive	45 (71.4)	11 (45.8)	56 (64.4)
Seronegative	18 (28.6)	13 (54.2)	31 (35.6)
BCG scar			
None	7 (11.1)	3 (12.5)	10 (11.5)
One	47 (74.6)	21 (87.5)	68 (78.2)
Two	6 (9.5)	0 (0)	6 (6.9)
Dubious	3 (7.8)	0 (0)	3 (3.4)

HCSDL: household contact of student diagnosed with leprosy; PGL-I: phenolic glycolipid-I.

TABLE IV

ELISA results

	Healthy students (n = 1,529)	Students diagnosed with leprosy (n = 63)	Healthy HCSDL (n = 232)	HCSDL diagnosed with leprosy (n = 24)	Private school students (n = 45)	Endemic control (n = 14)	Non endemic control (n = 3)	PB/RU (n = 10)	MB/RU (n = 41)
Median OD (IQR)[a]	0.286 (0.313)	0.411 (0.400)	0.274 (0.323)	0.240 (0.211)	0.187 (0.152)	0.113 (0.097)	0.077 (0.087)	0.523 (0.398)	1.586 (2.434)

a: optical density (OD) read at 490 nm. Cut-off for positivity = 0.295 OD; HCSDL: household contact of student diagnosed with leprosy; IQR: interquartile range; MB: multibacillary; PB: paucibacillary; RU: reference unit.

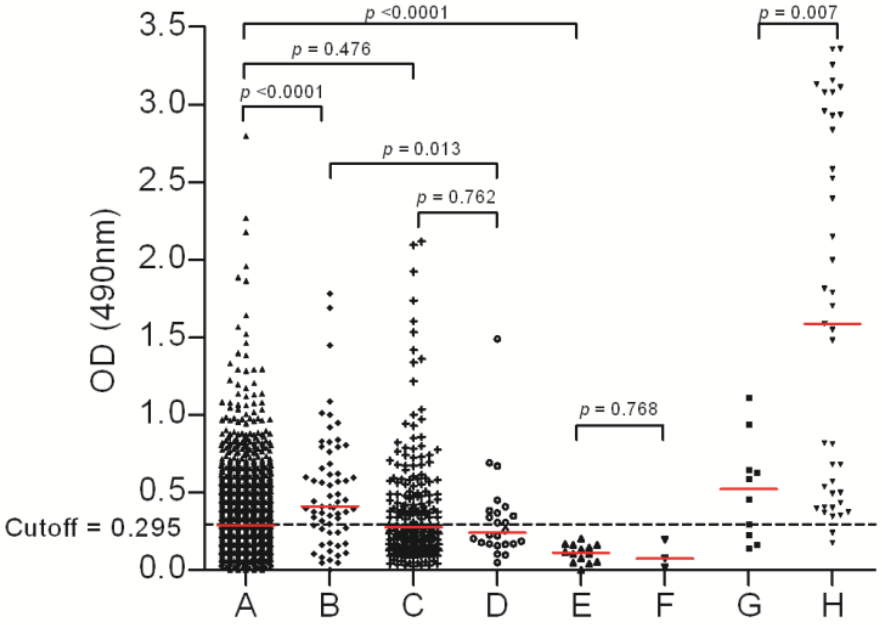

Fig. 3: levels of anti-phenolic glycolipid-I IgM from different groups: A: healthy students (n = 1529); **B:** students diagnosed with leprosy (n = 63); **C:** healthy household contacts (HCCs) of students with leprosy (n = 232); **D:** HCCs of students with leprosy that were also diagnosed with leprosy (n = 24); **E:** endemic controls (ECs) (n = 14); **F:** non-ECs (n = 3); **G:** paucibacillary patients from the leprosy reference unit (RU) (n = 10); **H:** multibacillary patients from the leprosy RU (n = 41); OD: optical density.

Regarding the degree of physical disability, there were no differences in the anti-PGL-I levels between those with grade 0 (median OD = 0.376; IQR = 0.410) and those with grade 1 or 2 (median OD = 0.301; IQR = 0.778) (p = 0.941). In addition, we did not find a significant association (p = 0.709) between the prevalence of seropositivity and BCG vaccination status (presence or absence of a BCG scar).

Amongst the study participants, 42.5% of the male students and 53.3% of the female students were seropositive (p < 0.0001). The median OD for the males was 0.256 (IQR = 0.262) and the median OD for the females was 0.323 (IQR = 0.354) (p < 0.0001), but the number of leprosy cases did not differ by gender (p = 0.792).

Thirty-four of the visited schools were in urban zones and three schools were in rural zones; we examined 1,428 urban students (54 new cases) and 164 rural students (9 new cases). There was no significant difference between the proportions of new cases detected in the two areas (p = 0.288). The level of anti-PGL-I was higher in the urban area (median OD = 0.301; IQR = 0.323) than in the rural area (median OD = 0.220; IQR = 0.276) (p < 0.0001) and the prevalence of seropositivity was also higher in urban areas (urban = 50.7%; rural = 32.3%; p < 0.0001).

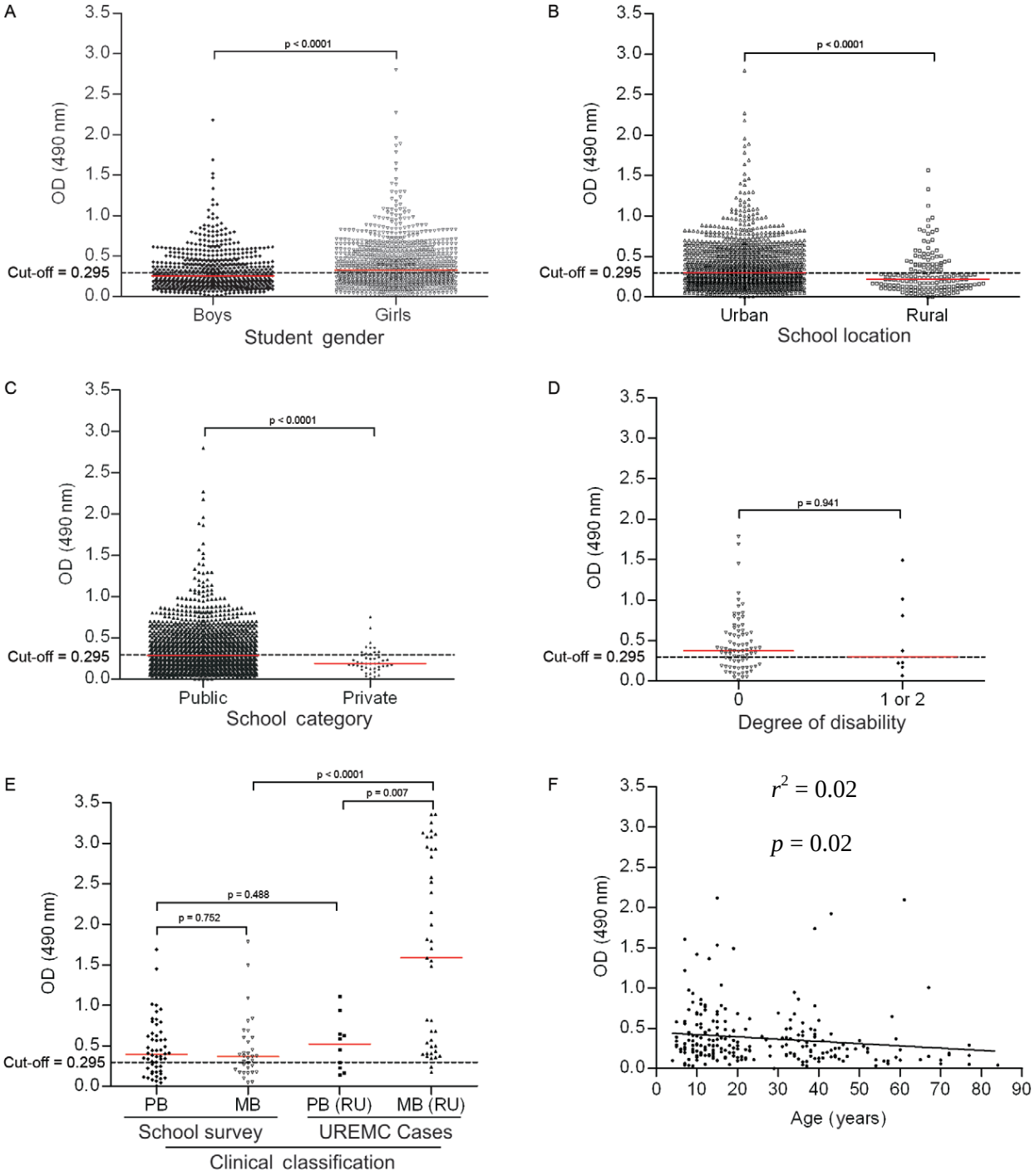

Fig. 4A: levels of anti-phenolic glycolipid-I (PGL-I) IgM according to the students gender; **B:** region of the schools; **C:** category (public or private); **D:** there was no significant difference according to the degree of physical disability; **E:** differences between cases detected during school survey [paucibacillary (PB) and multibacillary (MB)] and cases referenced to the reference unit (RU); **F:** correlation between the level of anti-PGL-I and the age of the household contacts of student diagnosed with leprosy; OD: optical density; UREMC: Dr Marcello Candia Reference Unit in Sanitary Dermatology.

Twenty newly diagnosed leprosy cases (23%) reported that they had experienced starvation (a full day without meals) at least once because they lacked the resources to buy food, 77 (89%) had family incomes of up to twice the Brazilian minimum wage, 73 (84%)

were receiving some type of governmental financial assistance, such as a family allowance or retirement benefits, and 59 (68%) lived in a house with more than two people per bedroom. Additionally, 54.5% of the newly diagnosed students stated that they were aware of previous contact with at least one person affected by leprosy.

Discussion

From 1991-2010, 88,805 new leprosy cases were diagnosed in PA alone (The National Notifiable Diseases 2012 - portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm ?idtxt=31200). Considering that there were approximately two million students enrolled in public schools in 2009 (Brazilian Institute for Geography and Statistics - ibge.gov.br/ estadosat/), we examined only 0.08% of all the students. If the data we collected were extrapolated to the entire population of SC, there may currently be approximately 80,000 undiagnosed leprosy cases amongst PA students. According to the World Health Organization, the peak age range for the onset of leprosy is 20-30 years (WHO 2009). However, while the diagnosis may occur during this period of life, the onset may occur earlier and the patient may be sick for a long time. In PA, almost half of the patients have been diagnosed between the ages of 20-60 years and in the last 20 years, an average of 4,400 patients were diagnosed each year. If we assume that the 80,000 currently undiagnosed leprosy cases will be detected in the next 20 years, we may maintain this average for a long time.

In fact, these data may be corroborated by the high proportions of anti-PGL-I-positive SC, from 28.7-66.5%. These figures are similar to the prevalence of subclinical infection detected by Dayal and Bharadwaj (1995) amongst healthy children who were close contacts of PB and MB LPs (61% and 75%, respectively). Although these numbers are high compared with those of other studies involving the general population, there is little surveillance of leprosy amongst SC. Some studies report lower seroprevalence rates, even in highly endemic regions (Cartel *et al*. 1990, Bührer-Sékula *et al.* 2008), whereas other studies report high seroprevalence rates that are compatible with the high incidence rate of the surveyed population (Abe *et al.* 1990, Van Beers *et al*. 1999) and corroborate our results.

The other interesting findings, already presented in other studies (Fine *et al*. 1988, Krishnamurthy *et al*. 1991, Bakker *et al*. 2004), are the higher levels of anti-PGL-I seropositivity amongst children and young adults compared with older adults (i.e., an inverse

correlation with age) and amongst girls compared with boys. There is no definite explanation for these results, but generally, IgM antibody levels vary with age and they are consistently higher in females than in males at every age (Oskam *et al*. 2003). Moreover, the difference in seropositivity between the urban and rural SC may be due to the poorer living conditions in urban areas, where the students live in more crowded houses and neighbourhoods and are more susceptible to food shortages. These observations, along with the high seropositivity rate amongst students in public schools, led us to question whether higher income students would have the same results. As expected, the prevalence of anti-PGL-I seropositivity amongst SC in private schools was significantly lower than the prevalence in public schools, confirming the strong correlation between leprosy and poverty.

Although the seroprevalence rate is high, we must remember that *M. leprae* is a highly infective, but low pathogenic bacterium that causes disease in only 10-20% of all infected people, considering a positive lepromin test in approximately 80% of the population protected by the "N-factor of Rotberg" (Rotberg 1989). According to our data, almost half of the SC in public schools have subclinical infections and respond by producing anti-PGL-I, which can be used as a marker of *M. leprae* dissemination into the community (Baumgart *et al.* 1993). Of the two million students enrolled in PA public schools, approximately one million may be positive for anti-PGL-I. A total of 15% of the healthy students in our sample had an anti-PGL-I titration that was two times higher than our threshold for positivity. If 5% of one million students become ill in the coming years, we will have an additional 50,000 new patients in the future, joining the 80,000 individuals who are currently ill. Some of these patients will experience mild signs and symptoms (e.g., single-lesion PB leprosy) and they may never become registered cases if their leprosy heals spontaneously, as described in the literature (Jesudasan & Christian 1985).

In fact, when examining the same population months after an initial screening for anti-PGL-I, as we did in the county of Oriximiná, in the western region of PA (Salgado *et al.* 2012), we found a high number of new leprosy cases amongst families with anti-PGL-I-positive individuals who were previously undiagnosed. Many factors may contribute to this high hidden prevalence. The clearest indicator is the low rate of contact examination, approximately 40% in PA, which is considered to be precarious by the Brazilian Ministry of Health (MS/SVS 2009). Additionally, the low coverage of the population by the family health programme, with almost 60% of people lacking access to the system (Department of Health Care/Department of Primary Care 2012 - dab.saude.gov.br/historico_cobertura_sf.php), may

explain the high number of undiagnosed leprosy cases. Furthermore, the majority of the cases we diagnosed were PB and many of them had only one lesion, sometimes a slightly hypochromic macule that was not recognised by the patient or family as a lesion. The family health programme team may be trained to detect well-established leprosy cases with clear symptoms, but the team may not be trained to diagnose cases in the early stages, such as those that we detected in this study.

Interestingly, the seropositivity rates amongst the PB and MB patients in our sample were almost the same. By contrast, the PB patients diagnosed at UREMC had a slight, but not significant increase in anti-PGL-I levels, while the UREMC MB patients had a significant increase in the median level of anti-PGL-I. These results confirm the early diagnosis because we detected more PB cases than MB cases and because the MB cases were mostly BT patients.

Surveys to detect leprosy among SC are not new. In 1947, researchers in British Guiana examined 42,811 students and found 94 (0.21%) new cases. The author proposed that surveying schools "should become a permanent part of the leprosy public health program" and concluded that without the study, the early cases might not have been identified until they were more advanced and difficult to cure (Wharton 1947). In a study similar to ours that was performed in India, Bhavasr and Mehta (1981) demonstrated that school surveys and contact examination of children could be considered to be a useful, inexpensive and rapid method for detecting leprosy cases in the community. In that study, visits to the homes of 24 new student cases revealed a family history of leprosy for 50% of the affected students. Similarly, Thirumalaikolundusubramanian and Prince (1983) examined 6,731 primary SC in India and 173 (2.7%) had leprosy. Silva *et al.* (2007) detected 20 new leprosy cases during an active search of 14,653 students, a case detection rate of 136/100,000 students, in Buriticupu, a hyperendemic municipality in the state of Maranhão (Brazil). In a cross-sectional survey of 1,114 students 11-20 years old in four districts in Timor-Lest, dos Santos *et al.* (2010) detected 17 (1.5%) new leprosy cases, which represents a case detection rate of 1,526/100,000 students. All five of these studies corroborate our findings. In addition to a high number of new student cases (63 cases out of 1,592 examined; 4%), we found 24 (9.4%) new cases amongst 256 HHCs of the students diagnosed with leprosy. This result clearly indicates that the contacts of infected students must be examined in addition to the students themselves.

Previously, we performed a study in the county of Oriximiná based on our serological data and found a high number of hidden cases (Salgado *et al.* 2012). Similarly, we performed a more thorough study in the county of Castanhal, where SC and family members of anti-PGL-I-positive individuals (identified 2 years ago) were examined. We identified more than 60 new cases of leprosy among approximately 400 examined individuals (unpublished observations), thus demonstrating the effectiveness of our strategy. In addition, we provided an in-service training session for the municipality health team workers.

Our results suggest that implementing school surveys for identifying leprosy cases is imperative in highly endemic areas. The advantage of our strategy is that it identifies early cases of leprosy (mainly PB cases) with no physical disabilities, thus preventing the spread of the infection in the community and breaking the chain of transmission that is responsible for the high incidence rate observed over time.

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Resultados adicionais

Seguindo a mesma metodologia de avaliação de contatos intradomiciliares de pessoas atingidas pela hanseníase descrita no Capítulo 2, apresento nas tabelas a seguir (A e B) um breve resumo dos resultados obtidos durante a avaliação realizada em todos os oito municípios incluídos neste estudo.

Dos 156 casos novos detectados entre os contatos intradomiciliares, 27 (17.3%) apresentavam algum grau de incapacidade física no momento do diagnóstico, sendo 18 (11.5%) com grau 1 e nove com grau 2 (5.7%).

Tabela A. Exame de contatos intradomiciliares em oito munícipios do Estado do Pará.

Município	Casos visitados	Contatos examinados	Contatos soropositivos (%)	Casos novos (%)	PB	MB (%)
Altamira	77	313	104 (33,2)	50 (16)	12	38 (76)
Breves	39	210	66 (31,4)	47 (22,4)	14	33 (70,2)
Castanhal	87	302	118 (39,1)	8 (2,6)	4	4 (50)
Marituba	79	248	142 (57,2)	4 (1,6)	1	3 (75)
Oriximiná	42	126	57 (45,2)	3 (2,4)	0	3 (100)
Paragominas	96	331	160 (48,3)	12 (3,6)	5	7 (58,3)
Parauapebas	66	252	93 (36,9)	11 (4,3)	5	6 (54,5)
Redenção	45	163	66 (40,5)	21 (12,9)	8	13 (61,9)
TOTAL	**531**	**1945**	**806 (41,4)**	**156 (8)**	**49**	**107 (68,6)**

Tabela B. Formas clínicas dos casos novos detectados.

Forma clínica	Número de casos (%)
Indeterminada	30 (19.2)
Tuberculóide	19 (12.2)
Dimorfa	105 (67.3)
Virchowiano	2 (1.3)
TOTAL	**156 (100)**

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CAPÍTULO 4

SPATIAL ANALYSIS SPOTLIGHTING EARLY CHILDHOOD LEPROSY TRANSMISSION IN A HYPERENDEMIC MUNICIPALITY OF THE BRAZILIAN AMAZON REGION.

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Abstract

***Background**:* More than 200,000 new cases of leprosy were reported by 105 countries in 2011. The disease is a public health problem in Brazil, particularly within high-burden pockets in the Amazon region where leprosy is hyperendemic among children.

Methodology: We applied geographic information systems and spatial analysis to determine the spatio-temporal pattern of leprosy cases in a hyperendemic municipality of the Brazilian Amazon region (Castanhal). Moreover, we performed active surveillance to collect clinical, epidemiological and serological data of the household contacts of people affected by leprosy and school children in the general population. The occurrence of subclinical infection and overt disease among the evaluated individuals was correlated with the spatio-temporal pattern of leprosy.

Principal Findings: The pattern of leprosy cases showed significant spatio-temporal heterogeneity ($p < 0.01$). Considering 499 mapped cases, we found spatial clusters of high and low detection rates and spatial autocorrelation of individual cases at fine spatio-temporal scales. The relative risk of contracting leprosy in one specific cluster with a high detection rate is almost four times the risk in the areas of low detection rate (RR = 3.86; 95% CI = 2.26 – 6.59; $p < 0.0001$). Eight new cases were detected among 302 evaluated household contacts: two living in areas of clusters of high detection rate and six in hyperendemic census tracts. Of 188 examined students, 134 (71.3%) lived in hyperendemic areas, 120 (63.8%) were dwelling less than 100 meters of at least one reported leprosy case, 125 (66.5%) showed immunological evidence (positive anti-PGL-I IgM titer) of subclinical infection, and 9 (4.8%) were diagnosed with leprosy (8 within 200 meters of a case living in the same area).

Conclusions/Significance: Spatial analysis provided a better understanding of the high rate of early childhood leprosy transmission in this region. These findings can be applied to guide leprosy control programs to target intervention to high risk areas.

Author Summary

Leprosy can lead to physical disabilities and deformities if not diagnosed and treated early. Even today, the disease affects more than 200,000 people per year, particularly the poorest people from developing countries, such as India, Brazil and Indonesia. Cases among children <15 years old have been used as an important indicator of recent transmission in the community. Recently, geographic information systems and spatial analysis have become important tools for epidemiology, helping to understand the transmission dynamics of several diseases. In this work, we determined the spatial and temporal distribution of leprosy in a hyperendemic municipality of the Brazilian Amazon region. In association with clinical, epidemiological and serological data of household contacts and school children in the general population, we further correlated the occurrence of subclinical infection and overt disease with the distribution of reported cases. We identified heterogeneity in the distribution of leprosy, with significant clusters of high and low detection rates. Our analysis revealed that children with leprosy or those harboring subclinical infection were in close proximity to spatial and temporal clusters of leprosy cases. These findings can be applied to guide leprosy control programs to target intervention more systematically to areas where the risk of leprosy is high.

Introduction

Leprosy is a chronic granulomatous infectious disease caused by *Mycobacterium leprae* that affects mainly the skin and peripheral nerves, which can lead to severe physical disabilities and deformities if not diagnosed and appropriately treated with multidrug therapy (MDT) in its early stages [1]. Although leprosy has been successfully suppressed in developed countries, 219,075 new cases in 105 countries were detected in 2011, as reported to the World Health Organization (WHO), with India, Brazil and Indonesia contributing 83% of all new cases [2]. Brazil, with 33,955 new cases detected in 2011 (according to the official numbers of the Brazilian Ministry of Health), has one of the highest annual case detection rates in the world (17.65/100,000 people), and the prevalence rate has yet to be reduced below the threshold of 1/10,000 people – the level at which leprosy would be considered "eliminated" as a public health problem [2].

The spatial distribution of leprosy in Brazil is heterogeneous: the more socioeconomically developed states in the south have achieved the elimination target, though high-disease burden pockets still remain in North, Central-West and Northeast Brazil [3]. These high-burden areas encompass 1,173 municipalities (21% of all Brazilian municipalities), approximately 17% of the total national population and 53.5% of all Brazilian leprosy cases detected between 2005 and 2007 [4]. Most of the areas with spatial clusters of cases are in the Brazilian Amazon, long recognized as a highly endemic leprosy area [3-6].

More than 7.5 million people live in the state of Pará, located in the Amazon region. This state is hyperendemic for leprosy both among the general population (51.1/100,000 people) and among children < 15 years old (18.3/100,000 people). These annual detection rates are much higher than the Brazilian averages of 17.6 and 5.2 per 100,000, respectively, in 2011 [7]. Moreover, these rates can be considered an underestimation of the real situation in Pará because only 42% of the population is covered by the primary health care service, responsible for leprosy control implementation and active case finding [8].

Leprosy in children is strongly correlated with recent disease and active foci of transmission in the community, particularly within families living in the same household, reflecting the inefficiency of local control programs for the timely detection of new cases and prompt MDT treatment, which would break the continuous spread of the disease [9]. Furthermore, the prevalence of undiagnosed leprosy in the general population has been

estimated to be much more in highly endemic areas, ranging from two to eight times higher than the registered prevalence [10-13]. A recent cross-sectional study of 1,592 randomly selected school children from 8 hyperendemic municipalities in Pará revealed that 4% were diagnosed with leprosy based on clinical signs and symptoms [14]. By means of an ELISA test to determine the serological titer of IgM anti-PGL-I (the *M. leprae*-specific phenolic glycolipid-I antigen), 48.8% of the students were positive, indicating immunological evidence of subclinical infection. Indeed, it was estimated that there may be as many as 80,000 undiagnosed leprosy cases among Pará students [14]. Moreover, it was demonstrated that 2.6% of the household contacts of those people affected by leprosy during the last 5 years in Pará also have leprosy and that 39% of them have a subclinical infection of *M. leprae* [15]. Individuals who have a positive antibody titer to PGL-I have an estimated 8.6-fold higher risk of developing leprosy than those who are seronegative [16].This scenario of a high hidden prevalence and of subclinical infection urges new studies and innovative interventional approaches.

Geographic information system (GIS) technology and spatial analysis have been applied to identify the distribution of leprosy at national, regional and local levels [4,17-19]. These new analytical tools are used to monitor epidemiological indicators over time, to identify risk factors and clusters of high endemicity and to indicate where additional resources should be targeted. The findings obtained by these methods are useful to increase the effectiveness of control programs, targeting areas of higher risk [20], which is particularly important in regions where available public health resources are scarce. GIS technology can also help to monitor the extent of MDT coverage and, as in the case of other classical tropical diseases or diseases of poverty, could play a major role in vaccine-efficacy or chemoprophylaxis trials [21].

In a previous cross-sectional study performed in June 2010 [15], we described the prevalence of undiagnosed leprosy and of subclinical infection with *M. leprae* among household contacts and school children in the municipality of Castanhal, located in the Brazilian Amazon region. In the present study, we applied spatial analysis techniques to identify the distribution of leprosy in this hyperendemic municipality. We describe the spatio-temporal distribution of leprosy and its correlation with the occurrence of new cases and subclinical infection among household contacts and, for the first time, among school children in the general population.

Material and Methods

Ethics Statement

This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethics Committee from the Federal University of Pará (protocol number 197/07 CEP-ICS/UFPA). All data analyzed were anonymized.

Study area

Our study was performed in Castanhal (1.29° S; 47.92° W), located 68 kilometers NE of Belém, the capital of the Brazilian State of Pará. The population size was 173,149 inhabitants in 2010, with 88.5% living in the urban area [22]. According to the municipal Secretary of Health, there were 633 newly detected leprosy cases from January 2004 to February 2010 and 132 in 2012 (24.2% among children < 15 years old). The annual case-detection rate in the general population was 73.7/100,000 inhabitants in 2012 (roughly four times the rate for Brazil as a whole); such a rate ranks the municipality as hyperendemic according to the parameters designated by the Brazilian Ministry of Health (≥ 40/100,000) and significantly higher than Pará's average (51.1/100,000) [7].

The residences of people affected by leprosy in the urban area of Castanhal and reported during the period of 2004 to February 2010 were georeferenced to produce detailed maps of the leprosy distribution. Additionally, spatial statistical methods were applied to identify patterns and possible risk factors associated with *M. leprae* infection.

Sampling design and methods

The residential addresses and demographic and epidemiological variables (age, gender, year of notification and operational classification of the cases notified during the defined period) were collected from the national notifiable diseases information system (SINAN). The exact location of each residence in the urban area was then georeferenced using a handheld GPS receptor (Garmin *e*Trex H, Olathe, KS, USA). However, not all addresses were mapped with a GPS because many areas of Castanhal are difficult to reach and unsafe. Those that could not be reached were geocoded using the Brazilian national address file for statistical purposes (http://www.censo2010.ibge.gov.br/cnefe/) provided by the Brazilian Institute of Geography and Statistics (IBGE); this database comprises all regular street addresses and its respective census tract identification around the country. In association

with a high-resolution satellite imagery base map (World Imagery, ESRI, Redlands, CA, USA), we identified the street location inside the specific census tract. This alternative mapping method can result in a loss of positional accuracy of up to 100 meters but allows matching a street address with its respective census tract (the spatial unit of analysis). IBGE was also the source for the base map of the 163 urban census tracts for this city and for the last Brazilian demographic census conducted in 2010.

Combining information from SINAN, IBGE and field-work mapping, it was possible to draw point pattern and kernel case density maps, calculate the number of cases and the annual case detection rate per census tract and identify areas with the highest risk of leprosy. Clinical, epidemiological and serological data from the evaluated household contacts and school children were obtained. The subjects were clinically assessed by an experienced leprologist to detect new cases, and their antibody titers of IgM anti-PGL-I were determined by ELISA as described previously [15]. We established an ELISA optical density of 0.295 as the cutoff for being considered seropositive. The subjects were also interviewed to identify their demographic and socio-economic characteristics. Detailed information about sampling and eligibility criteria can be found in Barreto *et al.* [15]. All maps were produced with the spatial reference SIRGAS 2000 UTM Zone 23S using ArcGIS 10 (ESRI, Redlands, CA, USA).

Data management and analysis

We performed spatial analyses by either grouping leprosy cases per census tract or using the georeferenced position. To minimize the effects of small numbers statistical instability, in addition to the calculation of the raw annual detection rate per census tract, we also calculated a spatially empirical Bayes (SEB) detection rate (based on a queen spatial weight matrix) to smooth the differences between contiguous areas, thereby increasing the stability of the data [23]. Global Moran's I spatial autocorrelation [24] was used to investigate the spatial clustering of the raw annual detection rate per census tract. The statistical significance was evaluated by comparing the observed values with the expected values under the complete spatial randomness assumption based on 999 Monte Carlo permutations for a significance level of 0.001. A Global Moran's I correlogram, a global index of spatial autocorrelation, was calculated to identify the range within which autocorrelation is significant and the distance at which it is highest. Local Moran's I [24], as a local indicator of

spatial association (LISA), was applied to identify the position of significant clusters of higher and lower detection rates.

Additionally, a Kulldorff's spatial scan statistic [24,25] was applied to detect the most likely cluster of cases per census tract considering the population at risk per area. The main goal of this analysis was to identify a collection of adjacent census tracts that were least consistent with the hypothesis of constant risk. This method defines circles, with radii ranging from the smallest distance between two tracts to one-half of the width of the study area. The method identifies a region formed by all tracts with respective centroids that fall within the circle and tests the null hypothesis of constant risk versus the specific alternative that the risks within and outside this region are different [19,24].

Leprosy transmission has been described as following a pattern called "stone-in-the-pond principle", whereby not only the household contacts of a leprosy case have an increased risk of infection but also the neighbors and the neighbors of neighbors are at higher risk when compared to the general population, with risk inversely decreasing with increasing distance [18,26,27]. Given that association among cases is considered to be a fine-scale process, we used areas with radii of 50, 100 and 200 meters around each of the cases detected during the study period to identify the spatial proximity of leprosy cases and students examined during the school-based surveillance.

Furthermore, a multi-distance global spatial cluster analysis (Ripley's global *k*-function) [28] was used to identify the spatial clusters of individual leprosy cases considering a range of distance from 50 m to 3,000 m, with distance lags of 50 m. This method considers all combinations of pairs of points and compares the number of observed pairs with the number expected at all distances, assuming a random distribution and taking into account the density of points, borders of the study area and sample size [29,30].

A local Knox test [31] to detect the spatio-temporal interaction of individual cases considering space lags of 50, 100 and 200 meters and time lags from 1 to 5 years was also applied. This method tests for possible interaction between the distance and time separating individual cases based on the number of case pairs found within a particular time-space window [32]. In our study we chose the space and time lags described above based on the average leprosy incubation period (3 to 5 years) and distances at which most of the houses of contacts are located [33]. The expected values of the test under a null hypothesis of random

case occurrence (in space and time) were estimated by performing 999 Monte Carlo simulations.

Nonspatial statistics, such as Chi-squared (χ^2) [34] and Mann-Whitney *U* tests [35], were applied to compare the proportion of seropositivity and the titers of IgM anti-PGL-I, respectively, among household contacts and school children according to the different levels of proximity to leprosy cases or hyperendemic areas. The relative risk of leprosy as a ratio of the probability of developing the disease based on exposure was also calculated for specific areas of the city according to the level of endemicity and compared to the risk in the general population (2 x 2 contingency table) [36].

The following software were used for the statistical analyses: Opengeoda 1.0 (GeoDa Center for Geospatial Analysis and Computation, Tempe, AZ, USA) to calculate the spatial weight matrix, spatially empirical Bayes detection rate per census tract and Local Moran's I (LISA); Clusterseer 2.3 (Biomedware, Ann Arbor, MI, USA) to perform the global Moran's I test, Kulldorff's spatial scan statistics and Knox space-time clustering test; Point Pattern Analysis (PPA) (San Diego State University, San Diego, CA, USA) to obtain the Global Moran's I correlograms; ArcGIS to calculate Ripley's *K*-function and BioEstat 5.0 (Sociedade Civil Mamirauá, Amazonas, Brazil) to perform the nonspatial statistics.

Results

Spatial analysis

According to the SINAN database, of the 633 newly detected leprosy cases in Castanhal between January 2004 and February 2010, 570 (90.0%) lived in the urban area and 46 (7.3%) in rural areas; residential addresses were unavailable (missing information) for 17 (2.7%), and these were not included in the analysis. Of those living in the urban area, 499 (87.5%) were mapped, half of them directly in the field using GPS and half via remote geocoding. The other 71 urban cases were not georeferenced due to inconsistent information regarding their residential addresses. Seventy-one percent of all cases were classified as multibacillary (MB).

Figure 1 illustrates the population density and spatial distribution of leprosy cases in the urban area of Castanhal and classifies the census tracts according to the level of endemicity, from low to hyperendemic, following the official parameters for the annual

detection rate. The smoothed detection rate (Figure 1D) produced a more refined map of leprosy compared to the raw rate (Figure 1C), decreasing the differences between the contiguous census tracts. A correlogram of the global Moran's I test showing the significant ($p < 0.01$) spatial autocorrelation of the census tracts with the high or low raw detection rate of leprosy per 100,000 people is shown in Figure S1. Taking into account the location of the census tract centroids, the most significant ($p < 0.01$) clustering distance was between 1 and 2 km (peaking at 1.5 km).

The kernel density estimation indicated large differences in the number of cases in different areas, ranging from 0 to 191 per square kilometer (Figure 2A). The highest case densities overlap the census tracts with high population densities, as shown in Figure 1A. Spatial statistics (LISA) detected a significant local spatial association (i.e., association between similar values) between the census tracts with high detection rates (high-high) and between areas with low detection rates (low-low) (Figure 2B). Kulldorff's spatial scan statistics also indicated the most likely cluster of leprosy cases in a specific area of the city (Figure 2C). Both statistics showed similarity in the clustering results in one of the areas but not in the others. Table 1 presents more detailed data regarding the specific regions represented in Figures 1 and 2, including the number of census tracts, population, mean individuals per house and relative risk of leprosy compared to the general population.

Based on our analyses, approximately 88,000 people, 57% of the total urban population of Castanhal, lived in census tracts classified as hyperendemic for leprosy based on the raw detection rate. The population density per square kilometer in areas of clustered high detection rates (Figure 2C, detected by Kulldorff's spatial scan statistics) was more than 2-fold higher than in areas with lower detection rates, and the risk of contracting leprosy in that cluster was almost four times the rate in the low-low areas indicated by LISA (RR = 3.86; 95% CI = 2.26 – 6.59; $p < 0.0001$). Using a Mann-Whitney test, we also observed that the household density (number of individuals per house) was significantly higher ($p < 0.0001$) in those residences with individuals affected by leprosy (mean = 5.0; SD = 2.6) than the city average (mean = 3.8; SD = 3.2). Hyperendemic areas (raw detection rate) showed the highest relative risk (RR = 3.69; 95% CI = 2.91 – 4.67), whereas we observed a decrease of 54% in the risk (RR = 0.46; 95% CI = 0.28 – 0.74) in the low-low areas (LISA test) compared to the general population. The Spatial Bayesian Smoothing of detection rates increased the number of census tracts classified as hyperendemic from 93 to 114. Using the raw and smoothed rates, we calculated the number of people whom we need to follow to detect one new case of

leprosy in a cohort, and we found that the number of those individuals nearly triples when the smoothed rate was used instead of the raw detection rate (Table 1).

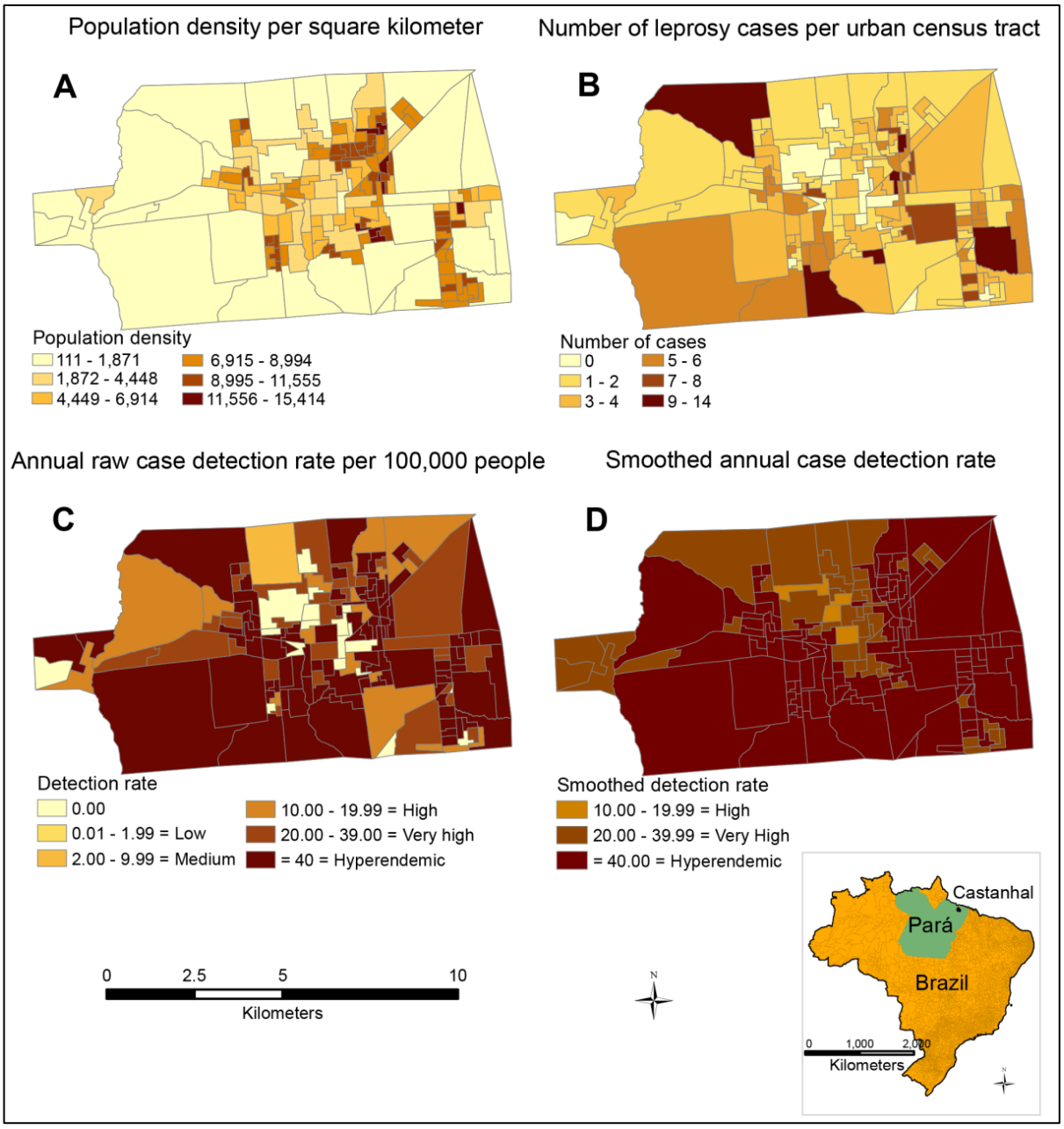

Figure 1. Population density and spatial distribution of leprosy in Castanhal. (A) Population density per km^2 in the urban census tracts. (B) Raw number of leprosy cases per census tract. (C) Number of cases normalized by the population of each census tract per year (annual raw case detection rate per 100,000 people), classifying areas according to their level of endemicity, from low to hyperendemic, according to official parameters. (D) Spatially empirical Bayes smoothed detection rate (based on a queen spatial weight matrix) to smooth the differences between contiguous areas.

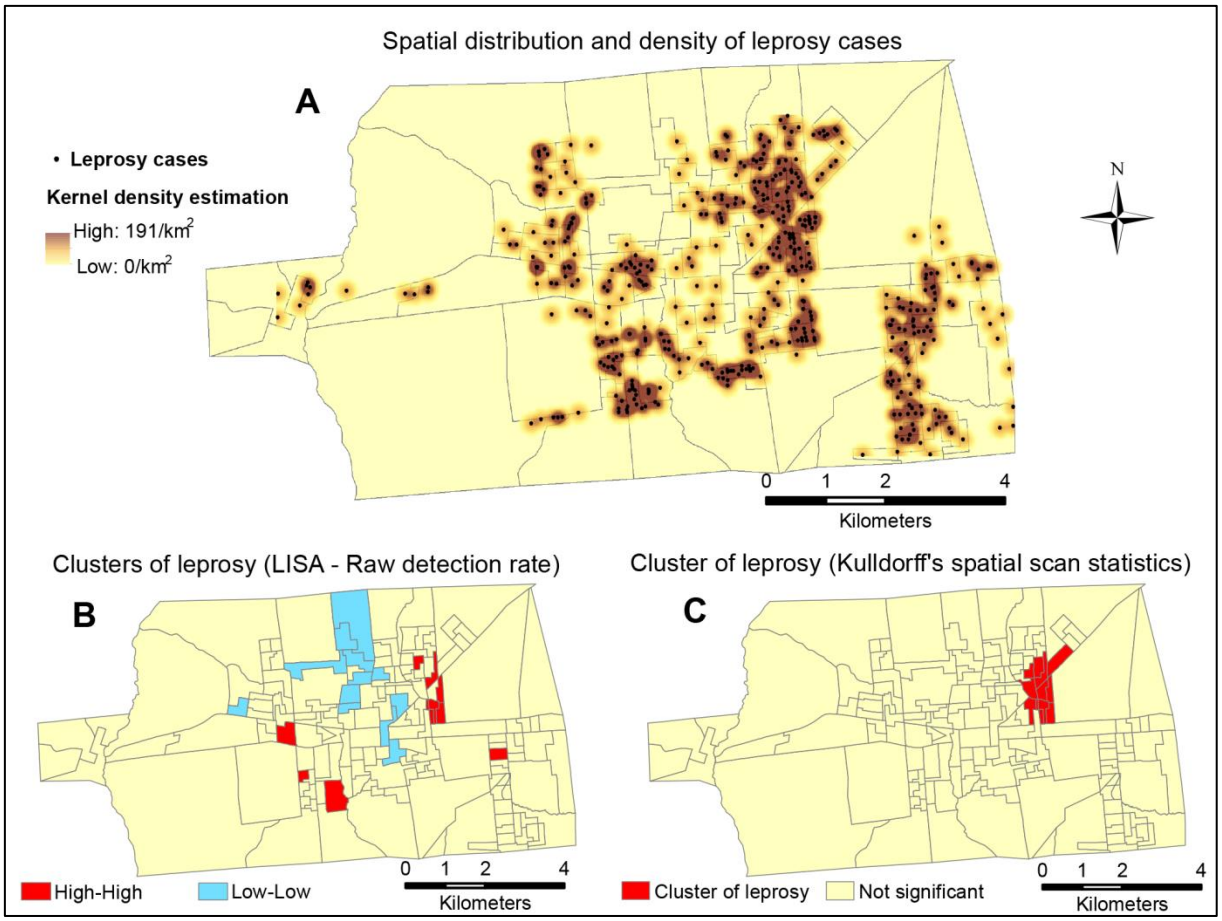

Figure 2. Clusters of leprosy in Castanhal. (A) The spatial distribution of individual leprosy cases overlying the respective Kernel density estimation layer, representing areas with a high and low density of cases per km2. (B) LISA test (local Moran's I) characterizing areas with a statistically significant ($p < 0.05$) positive spatial association according to the raw detection rate. The areas marked as high-high indicate a high rate in an area surrounded by high values of the weighted average rate of the neighboring areas, and low-low represents areas with a lower rate surrounded by lower values. (C) The most likely cluster of leprosy detected by the Kulldorff's spatial scan statistics ($p < 0.01$).

Table 1. Characteristics of the specific regions in the urban area of Castanhal.

	Number of census tracts	Total population	People per house Mean (SD)	People per km^2	Number of cases	Raw detection rate* Mean (SD)	Relative risk (95%CI)	*p*-value	Number of people to be followed to detect one case in a cohort
Hyperendemic areas of raw detection rate	93	88,333	3.8 (0.2)	6,847	416	75.9 (28.6)	3.69 (2.91 – 4.67)	<0.0001	295
Hyperendemic areas of SEB smoothed detection rate	114	109,307	3.8 (0.2)	6,584	395	58.2 (36.3)	1.53 (1.24 – 1.9)	<0.0001	834
Cluster detected by spatial scan statistics	11	10,472	3.7 (0.2)	9,536	63	97.0 (30.4)	1.97 (1.51 – 2.56)	<0.0001	345
High-high areas of raw detection rate (LISA)	10	8,756	3.8 (0.2)	8,777	49	90.2 (23.1)	1.79 (1.33 – 2.40)	<0.0001	400
Low-low areas of raw detection rate (LISA)	12	10,914	3.8 (0.2)	4,547	17	25.1 (16.7)	0.46 (0.28 – 0.74)	=0.0007	Decrease of 54% in the RR

*Annual detection rate per 100,000 people.
SEB = Spatially empirical Bayes.
LISA = Local indicator of spatial association (Local Moran's I).

Spatial analysis and leprosy in household contacts

A total of 302 household contacts were evaluated during previous visits to 88 residences of people affected by leprosy [15]. Sixty-three examined contacts (20.9%) lived in areas of clustered high detection rates of leprosy based on LISA and Kulldorff's spatial scan statistics. However, there were no significant differences in the serological titer of IgM anti-PGL-I (p = 0.481) or in the percentage of seropositivity (p = 0.471). Of the 8 new cases detected among household contacts, 2 lived in areas of clusters of high detection rate and 6 in hyperendemic census tracts outside the clusters.

Spatial analysis and leprosy in children

Approximately 10% of the cases from 2004 to 2010 in Castanhal involved children < 15 years old. Of the 499 mapped cases, 44 were children, with 36 (82%) living in hyperendemic areas of the city. Four public schools (two elementary and two high schools) located in different peripheral neighborhoods were also visited to evaluate a randomly selected sample of students (n = 188) for the clinical signs and symptoms of leprosy and also for subclinical infection by serological assessment of anti-PGL-I titer by ELISA assay. All four schools visited were in the hyperendemic census tracts: 134 of 188 (71.3%) examined students lived in hyperendemic areas (Figure 3); 41 (21.8%) were residing within 50 meters of at least one leprosy case; and 120 (63.8%) and 178 (94.7%) were dwelling less than 100 or 200 meters, respectively, from a known case. We did not observe significant differences in the levels of IgM anti-PGL-I (p = 0.894) or in the seropositivity between these three levels of proximity (p = 0.455). One hundred and twenty five students (66.5%) were seropositive; 9 (4.8%) were diagnosed with leprosy (8 within 200 meters of a case, 7 within 100 meters and 2 within 50 meters). Additionally, when the students diagnosed with leprosy were visited at home, 3 more cases were detected among their relatives, and 7 tested positive for anti-PGL-I.

Multi-distance point pattern analysis (Ripley's k-function) identified a significant clustering of reported individual cases, starting at a distance of 50 meters (Figure S2). To assure that the remotely mapped leprosy cases (geocoded) did not affect the results of the point pattern analysis as a function of the potential loss of accuracy of this method (up to 100 m), we also performed a multi-distance point pattern analysis (Ripley's global k-function) considering only the cases mapped using GPS directly in the field, revealing the same significant pattern of spatial clustering. Additionally, using the Gi*(d) test, we observed no

significant clustering pattern in the underlying population considering the variables: total population per census tract, mean people per house and density of people per square kilometer.

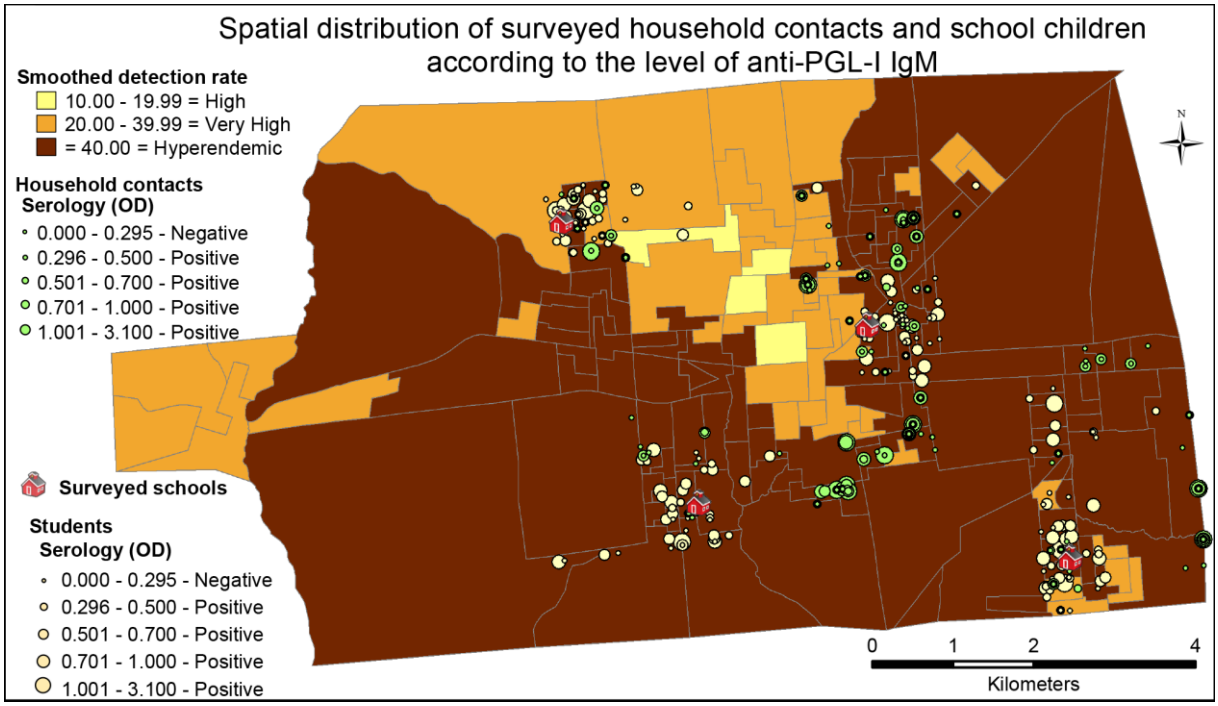

Figure 3. Spatial distribution of surveyed household contacts and school children. The spatial distribution of surveyed household contacts and school children according to their level of antibodies compared to the level of endemicity of the different census tracts.

Using the Knox test, we determine that the reported cases were also clustered in space and time and, as expected, frequently among household contacts, as was observed in 21 houses in which more than one case (2 or 3) shared the same residence. Table 2 displays the results of the Knox space-time clustering analysis for the leprosy cases based on different space-time lags. We identified up to 406 of 499 (81.3%) mapped cases that were near other cases in both space and time, summarizing 663 space-time links in 63 clusters. Figure 4 is an expanded view of a specific region identified as a cluster of leprosy and surrounding area, showing the space-time links among cases (100 meters over a 3 year period) and the spatial relationship with a surveyed school and seropositive students. All 6 school children (3.2%) with no clinical manifestations of leprosy who tested strongly positive for anti-PGL-I (ELISA optical density > 1.000), similar to that observed in multibacillary patients, were dwelling within 100 meters of at least one leprosy case, consistent with the uncovered and upcoming spatio-temporal associations.

Table 2. Knox space-time clustering analysis for leprosy cases*

Space-time lag (meter-years)	Number of space-time links	Number of cases	*p*-value (999 Monte Carlo simulations)
50 - 1	56	91	0.013
50 - 2	69	108	0.012
100 - 1	176	226	0.010
100 - 2	224	259	0.012
100 - 3	270	289	0.019
100 - 4	296	307	0.011
200 - 2	663	406	0.009

*Only statistically significant space-time lags are shown here ($p < 0.05$). Total number of analyzed cases = 499.

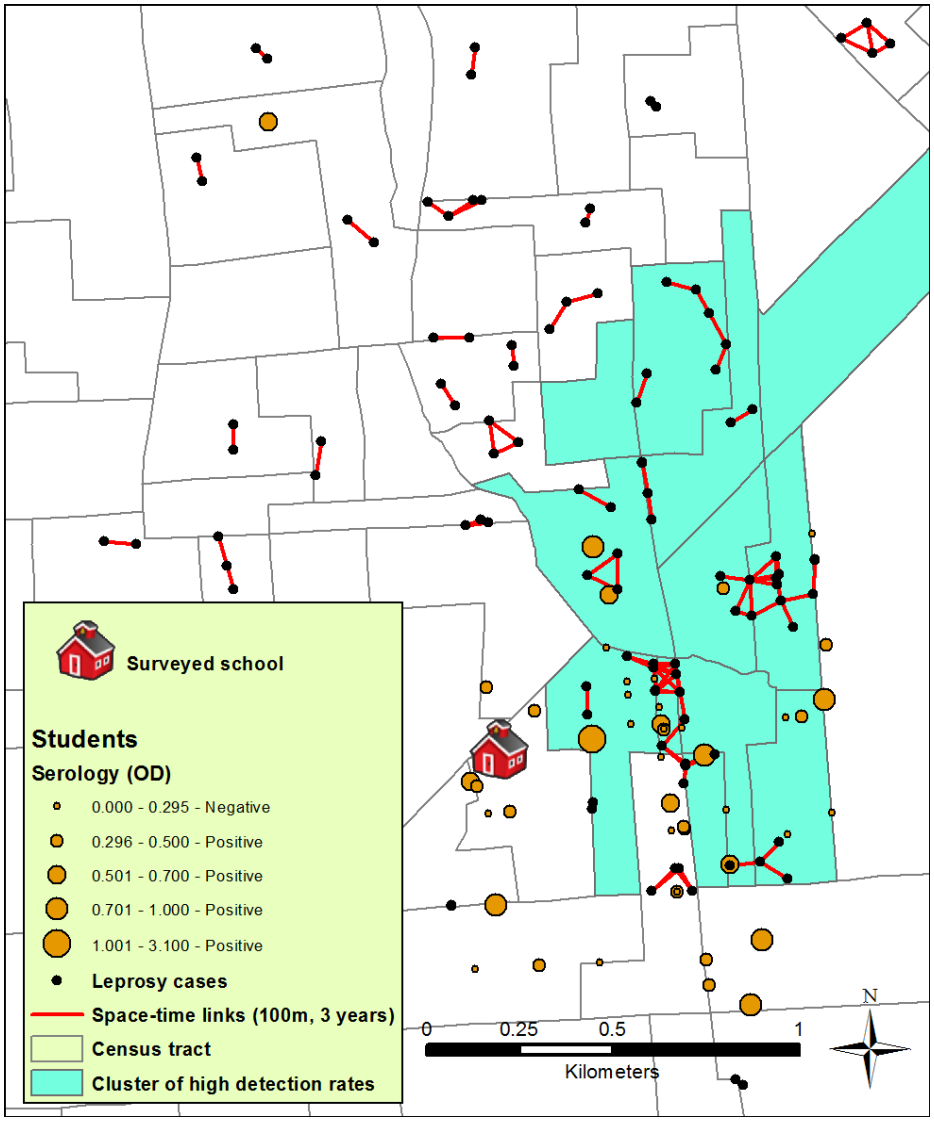

Figure 4. Space-time links among cases and proximity to students. An expanded view of a specific region identified as a cluster of leprosy (see Figure 2C, Kulldorff's spatial scan statistics), showing the space-time links among cases and the spatial relationship with a surveyed school and seropositive students.

Discussion

The pattern of leprosy cases reported from 2004 to 2010 in Castanhal showed significant spatio-temporal heterogeneity, and we found spatial clusters of high and low detection rates in the urban area. Using spatial global tests, we were also able to determine that the spatial autocorrelation of both the raw detection rate at the census tract level and of individual cases occurred at fine temporal and spatial scales. According to an analysis of the spatial pattern of serological data obtained by testing students, we ascertained that children with a high serological titer of anti-PGL-I were in close proximity to spatial-temporal clusters of leprosy cases. These findings can be applied to guide leprosy control programs to target intervention to locations with the highest risk of leprosy. De Souza Dias and colleagues [20] described the first application of GIS tools to direct active case-finding campaigns at a fine geographic scale in Brazil [20] and were able to target hot spots, resulting in the enhanced detection of new cases in addition to realizing important cost reductions for leprosy control activities.

The surprisingly high previously undiagnosed prevalence of leprosy and of subclinical infection with *M. leprae* among school children can be explained by the close proximity of these students' homes to detected cases. It has been shown that, in addition to household contacts, people living in the vicinity of a leprosy case and their social contacts have a higher risk of infection [18,26,37]. In fact, because *M. leprae* is highly infective but has a low pathogenicity, most people who harbor a subclinical infection will never develop clinical signs and symptoms of leprosy; indeed, only about 10% of all infected individuals eventually develop leprosy symptoms [38]. Due to the slow doubling time (13 days) and long incubation period prior to the onset of frank disease symptoms (3-5 years or longer), it is likely that many hidden cases exist, although serological responses to some protein antigens have been shown to predict disease progression up to a year prior to diagnosis [39-43]. It has been well-established that the titer of anti-PGL-I IgM antibody is directly correlated to the bacillary index, and that very high titers to PGL-I and certain protein antigens, such as LID-1 and Ag85B (ML2028) indicate a greater risk of developing disease [27,40,43]. The main challenge is to discover which biomarkers of infection serve as the best predictors of who will succumb to disease. Accordingly, performing targeted surveillance on individuals living in high endemic areas and following individuals with a high titer of anti-PGL-I is a strategy that must be implemented to perform early diagnosis, prevent physical disabilities and break the chain of transmission.

A number of serological surveys have shown that the rate of anti-PGL-I seropositivity in endemic settings correlates well with leprosy incidence in the community [44,45]. All of the surveyed schools in this study were located in the hyperendemic census tracts of the city. This finding explains the absence of significant differences in the seroprevalence or in the titer of antibodies in the students based on a geographic location, given that nearly all (95%) of them were living within 200 meters of a detected leprosy case.

As observed for the students, there were no differences in the titer of anti-PGL-I or seroprevalence among the household contacts living inside or outside a cluster of cases. This is also not surprising, given that, even outside a cluster, all household contacts were living in very high or hyperendemic areas and that the most likely source of *M. leprae* is a close contact that shares the same house or room. Indeed, when 942 students and 58 teachers from Castanhal were asked if they knew a person affected by leprosy, 17.7% of the students and 53.4% of the teachers answered in the affirmative. In addition to this proximity, those harboring a subclinical infection could be a potential source of contamination to others [46], rendering such frequent-, intensive- and close-social-contact environments, such as households and schools, as locations that are favorable for *M. leprae* transmission.

Considering its total area, the Brazilian Amazon region has the lowest population density (4.12 individuals/km^2) in the country but the highest number of people per household (3.97). This is a direct result of poverty, which compels relatives and others to live together for long periods of time, especially young married couples and their children, typically under precarious sanitation conditions. Furthermore, the average household density was even higher in the residences with a leprosy case (5.0), and, for purpose of comparison, this population density per square kilometer within the cluster of leprosy (9,536/km^2 – Figure 2C) was as high as New York City (10,429/km^2 - http://www.census.gov). Within the context of the wide recognition that high levels of crowding facilitate the transmission of infectious disease [47], it is reasonable to suggest that improvements in the socioeconomic status and living conditions should be part of the overall leprosy control strategy.

The introduction of GIS to leprosy epidemiology brought new insight to the concept of defining contacts based on relative distance. The importance of performing periodic surveillance among household contacts and including different classes of social and neighboring contacts has been highlighted by several authors [33,37,48]. Bakker and colleagues [18] observed increased subclinical infection for contact groups living ≤ 75 meters

of anti-PGL-I-positive leprosy patients. Another report described that 92% of the dwellings of contacts were within a distance of 100 meters of the index patient [33]. For this study, we selected radii of 50, 100 and 200 meters and observed significant space-time clusters within all of these distances. Leprosy was also found to exhibit a clustered spatio-temporal pattern in an analysis of more than 11,000 cases for a period of 15 years in Bangladesh [49], with most clusters having a duration of 1 or 2 years and one cluster a 4-year time span. In our study, we observed significant spatio-temporal clustering, even within a very fine geographic scale, which is compatible with direct human-to-human transmission. Most of the students diagnosed with leprosy (8 of 9) lived in close proximity to previously detected cases.

A spatially empirical Bayes smoothed case detection rate has been used in leprosy studies to smooth the random variations in small areas with few people (where small variations in the number of cases results in dramatic changes in disease rates) and to enhance the visualization of spatial patterns [17,50-52]. Smoothing is also a way to estimate uncertain values for areas with no registered cases, areas where disease is not necessarily absent but may not have been detected due to operational limitations. Smoothing produced a clearer map of leprosy in Castanhal but increased the estimate of the number of people to be followed to detect one case. We agree with Odoi and colleagues [23] that the results obtained using spatial smoothing need to be treated with caution because they can mask large differences between neighboring regions.

Given that 71 (12.5%) cases in the urban area were not mapped and analyzed in this study and considering the high prevalence of undiagnosed cases in Castanhal, our data strongly supports the notion that many more individuals than those presented here, including many children < 15 years old, are currently infected with *M. leprae.*

In the last decade, spatial analysis and GIS have become important tools for understanding leprosy transmission dynamics in resource-poor countries. Different spatial statistical methods have been applied, including Kulldorff's spatial scan statistics [53] and global and local Moran's I indices of spatial autocorrelation [54]. However, because all spatial statistics have advantages and disadvantages, more than one method may be necessary to analyze the data and to enable decision makers to determine the priority areas for targeting control activities. Overlaying individual case point maps over high-resolution satellite images from high-risk areas (not shown here to protect the individual addresses) provides a clear visualization of the leprosy problem and can help to optimize active case-finding strategies

and plan further clinical, epidemiological and prophylactic studies. Additionally, combining clinical, epidemiological, serological and spatial data provided a better understanding of the transmission dynamics of leprosy at fine spatial scales and indicated high rates of childhood leprosy transmission within hyperendemic cities of the Brazilian Amazon region.

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Supporting Information

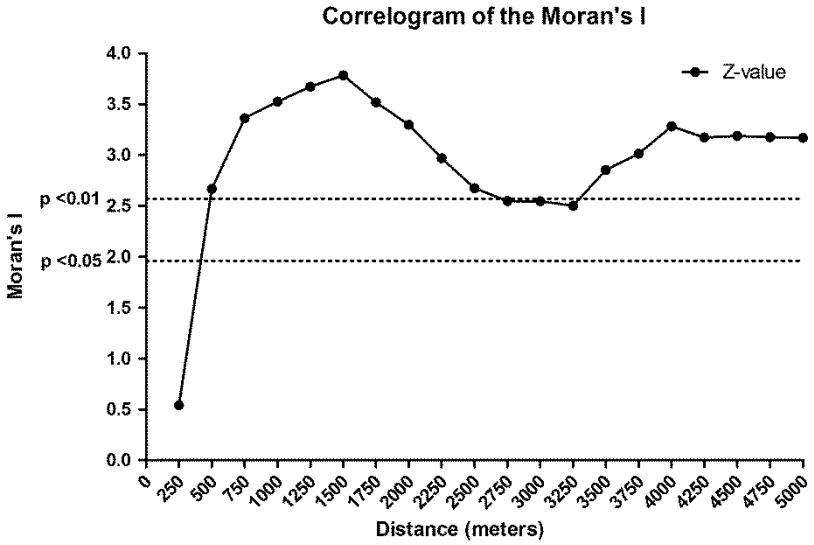

Figure S1. Correlogram of global Moran's I for the detection rates of leprosy by census tract in the urban area. Significant ($p < 0.01$) spatial autocorrelation of the census tracts with the high or low raw detection rate of leprosy per 100,000 people. Taking into account the location of the census tract centroids, the most significant ($p < 0.01$) clustering distance was between 1 and 2 km (peaking at 1.5 km).

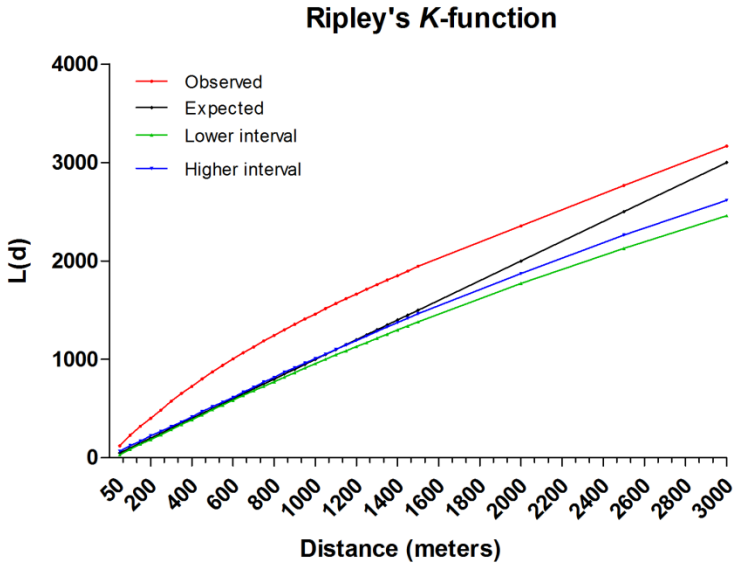

Figure S2. Multi-distance spatial cluster analysis (Ripley's k-function). There is significant clustering of individual cases starting at a distance of 50 meters ($p < 0.01$), indicating that cases tend to be detected in close spatial proximity.

CAPÍTULO 5

SPATIAL EPIDEMIOLOGY ASSOCIATED WITH SEROLOGIC COHORT AS A NEW STRATEGY FOR EARLY DIAGNOSIS OF LEPROSY.

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(A ser submetido)

Abstract

***Background**:* Leprosy remains an important public health problem in some specific high-burden pockets areas, including the Brazilian Amazon region, where leprosy is hyperendemic among children, indicating undiagnosed active foci of infection in their community.

Methodology: Based on the spatial epidemiology of leprosy in a hyperendemic municipality of the Amazon region, we selected 2 elementary schools placed in areas at most risk (cluster of leprosy or hyperendemic census tract) to clinically and serologically evaluate randomly selected school children (SC). Moreover, we followed-up anti-PGL-I seropositive or seronegative individuals and household previously evaluated in order to compare the incidence of leprosy in both groups after a period of 2 years.

Principal Findings: Eleven (8.2%) out of 134 SC of high risk areas were early detected with leprosy. The difference is statistically significant ($p = 0.04$) when compared to our previous findings in randomly selected schools (63/1,592; 3.9%). Otherwise, 22.3% and 9.4% of seropositive and seronegative individuals, respectively, developed leprosy after 2 years follow-up ($p = 0.02$). The odds of developing overt disease in seropositive people were 2.7 times that of negative ones (95%CI = 1.29 – 5.87; $p = 0.01$), pointing out that it is necessary to follow-up just 8 seropositive people to detect one case in two years. The odds of clinical leprosy was also higher in "positive houses" compared to "negative houses" (OR = 2.6; 95%CI = 1.18 – 5.91; $p=0.02$), indicating that it is necessary to follow-up 10 people living in households with at least one seropositive dweller to detect one new case in a period of two years.

Conclusions/Significance: School based screening in areas at most risk of leprosy indicated by spatial analysis in each endemic municipality and targeted household and individual continuous surveillance based on serologic data should be applied to increase the early detection of new cases.

Author Summary

Even today, leprosy affects more than 200,000 people per year, particularly the poorest communities from developing countries, such as India, Brazil and Indonesia. Recently, spatial epidemiology has become an important tool for public health, helping to understand the transmission dynamics of several diseases and guiding targeted intervention in areas at most risk. In this study, we performed school children active surveillance in 2 schools placed in high risk areas identified by the spatial analysis of leprosy distribution in a hyperendemic municipality of the Brazilian Amazon region. Additionally, based on a previous study among household contacts of leprosy patients and children in the general population, we followed-up subjects with or without an immunological evidence of subclinical infection (antibodies against *Mycobacterium leprae*) to identify the outcome 2 years after the first evaluation. Our analysis revealed that school based screening in areas at most risk of leprosy indicated by spatial analysis and targeted household and individuals' continuous surveillance based on serologic data increase the early detection of new leprosy cases.

Introduction

Although the World Health Organization (WHO) elimination target has been achieved in 2000, with a global prevalence rate of < 1 case/10,000 people, leprosy remains an important public health problem in some specific high-burden pockets areas [1]. From the most recent global statistics, 220,810 (95%) of new leprosy cases were reported from only 16 countries, and of them, India, Brazil and Indonesia contributing with more than 80% of all new cases [2].

Brazil has one of the highest annual case detection rates in the world (17.2/100,000 people), with 33,303 new cases reported in 2012 [2]. Despite the recent Brazilian economic growth, large pockets of poverty remain, especially in the North, Central-West and Northeast of the country, in which leprosy is hyperendemic and underdiagnosed [3,4]. Approximately half of the Brazilian cases were detected in high-burden municipalities that encompass only 17% of the total national population [5].

The problem is historic in the state of Pará, in the Brazilian Amazon region, north of the country, where roughly 80,000 new cases were reported during the last 20 years, making the 2012 annual case detection rate in Pará (50/100,000) the triple of the national average (17/100,000) according to official numbers of the Brazilian Ministry of Health. The transmission is still ongoing, once leprosy is also hyperendemic among children below 15 years old, and indicator of active foci of infection in the community [3,6]. Additionally, the low coverage of the population by the family health program, which is in charge for detecting and treating leprosy cases, with almost 60% of people lacking access to the system (http://dab.saude.gov.br/portaldab/historico_cobertura_sf.php), may explain the high number of undiagnosed leprosy cases recently discovered in Pará [3,6]. We estimate that there are approximately 80,000 cases among the 2,000,000 students of public schools to be diagnosed in Pará, many of them in hard to reach areas.

There is no laboratory test that detects all forms of leprosy, but some biomarkers of infection, disease progression and treatment efficacy have been developed since the isolation and characterization of phenolic glycolipid-I (PGL-I) in the eighties, a species-specific antigen from the *M. leprae* cell wall [7,8]. Various studies have demonstrated that serology could potentially be used to detect antibodies against PGL-I to classify patients for treatment

purposes, monitor cases, identify the risk of relapse and identify the household contacts (HHC) of leprosy patients who are most at risk of contracting the disease [9].

Anti-PGL-I seropositivity is also a marker of subclinical infection in healthy subjects [10,11]. A positive test for anti-PGL-I IgM is associated with an 8.6-fold higher risk of leprosy in HHC and a 4.4-fold higher risk in non-contacts [12]. Our recent school-based surveys have found 48.8% seropositivity among students ranging from 6 to 20 years old in Pará, and 4% of the students were detected with overt disease during this cross-sectional study [3]. Additionally, it is believed that a healthy carrier might not only have subclinical infection, but may also be actively involved in bacilli transmission, disseminating it in endemic regions [13].

Place has been key dimension of epidemiology and public health for decades. The huge growth in spatial epidemiology seen recently is facilitated by improved accessibility of computer-based geographic information systems (GIS) and personal computing improvements in processing speed, and user-friendly applications, what have placed spatial analysis within reach of a large number of researchers and health policy-makers [14]. GIS technology and spatial analysis have been applied to identify the distribution of leprosy at national, regional and local levels [4,15-17]. These new analytical tools are used to monitor epidemiological indicators over time, to identify risk factors and clusters of high endemicity and to indicate where additional resources should be targeted. The findings obtained by these methods are useful to increase the effectiveness of control programs, targeting areas of higher risk [18], which is particularly important in regions where available public health resources are scarce. GIS technology can also help to monitor the extent of basic public health service coverage and, as in the case of other classical tropical diseases or diseases of poverty, could play a major role in vaccine-efficacy or chemoprophylaxis trials [19].

The WHO believes that if innovative case-finding methods are introduced to access areas and population groups which are difficult to reach, together with improved data management, an increase in detection of new cases can be expected [2]. Considering that our recent studies have found a very high rate of previously undiagnosed leprosy and subclinical infection in the state of Pará [3,6], and that leprosy can be spatially clustered in hyperendemic pockets, even considering a fine intra-town spatial scale (unpublished observations), the main objective of this study is to describe and evaluate a new strategy for early diagnosis of leprosy

cases based on an association of spatial epidemiology tools and the anti-PGL-I IgM serologic cohort.

Material and Methods

Ethics Statement

This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethics Committee from the Federal University of Pará (protocol number 197/07 CEP-ICS/UFPA). All data analyzed were anonymized.

Setting

Our study was performed in two municipalities of the State of Pará: Castanhal (1.29° S; 47.92° W) and Oriximiná (1.76° S; 55.86° W); the first is hyperendemic and the second is highly endemic for leprosy. Castanhal is located 68 km NE of Belém, the capital of Pará, with an easy access through paved road. Differently, considering a straight line, Oriximiná is 820 km W of the capital, with access only by airplane or days travelling by boat on the Amazon and Trombetas Rivers. Table 1 presents some relevant demographic and epidemiologic characteristics of the municipalities.

Table 1. Epidemiologic and demographic characteristics of the study area.

Municipality	Population (2010)[a]	Number of new cases detected (2006 to 2010)[b]	Annual new case detection rate per 100.000 people (2006 to 2010)[b]	Children among new cases of leprosy (2006 to 2010)[b]	Seroprevalence among students[c]	New cases detected among students[d]
Castanhal	173,149	380	44.4	35 (9.2%)	66.5%	4.8%
Oriximiná	62,794	68	22.3	5 (7.3%)	42.2%	4.4%

[a] Font: Brazilian Institute of Geography and Statistics (IBGE).
[b] Calculated from the Brazilian Ministry of Health online database – SINAN.
[c] Seroprevalence of anti-PGL-I IgM detected in our previous cross-sectional study carried out in 2010 [3].
[d] New cases detected based on clinical examination in our previous cross-sectional study [3].

Sampling design and methods

Based on our previous clinical and serologic cross-sectional studies carried out in Castanhal and Oriximiná in 2010 (T1), evaluating 427 HHC and 323 school children (SC) [3,6], we sampled those clinically healthy subjects that tested positive or negative to anti-PGL-I to be reexamined two years after the first evaluation (T2). In order to be followed up, the subject must have been living in the same urban area we registered at the beginning of the

study. In addition to those people evaluated in T1, we included others HHC that were found in the households at the moment of our second visit, even though they were not examined in T1. The sample size was determined by the number of people that we could survey in one week of field work trip in each municipality.

The subjects were clinically assessed by an experienced leprologist. Leprosy cases were diagnosed in the field on the basis of clinical signs, loss of sensation on the skin lesions, and presence of enlarged nerves. For operational reasons, slit skin smears was not performed. The cases were classified as indeterminate leprosy, as defined by the Madrid classification [20], if there was only a hypopigmented macule, but no detection of nerve involvement; or as one of the clinical forms defined by the Ridley and Jopling classification system [tuberculoid-tuberculoid (TT), borderline tuberculoid (BT), borderline-borderline (BB), borderline lepromatous (BL) or lepromatous-lepromatous (LL)] [21]. Cases of indeterminate and TT leprosy were classified as paucibacillary (PB) cases, while the other forms were classified as multibacillary (MB) cases. Primary neural leprosy was diagnosed if nerve enlargement was detected, but no skin signs were present. When only one nerve was affected, the case was classified as PB; two or more enlarged nerves defined the case as MB. The disability grading (DG), ranging from 0 to 2 (0 = no disability; 1 = loss of sensation; 2 = visible damage or disability) was also determined by clinical examination of the sensory-motor functions using a WHO standardized neurological evaluation [22].

The subjects' anti-PGL-I IgM antibody titers were determined by ELISA as described previously, using native PGL-I as the antigen [6]. The ELISA cutoff to be considered seropositive was established as an optical density (OD) of 0.295, based on the average plus 3x the standard deviation of the test results from 14 clinically healthy people from the Amazon region. The subjects were also interviewed to identify their demographic and socio-economic characteristics. Detailed information about sampling and eligibility criteria for the first examination can be found in Barreto *et al.* [6].

Moreover, based on the spatial distribution pattern of leprosy cases, earlier described in Castanhal (unpublished observations), we selected 2 schools located in high risk areas, one in a cluster of leprosy and other in a hyperendemic census tract, in order to survey additional SC. We sent invitation letters to the parents of students of 3 or 4 classes, randomly selected by the director of each school (roughly 100 students), in elementary public schools, to attend a meeting with us, where they received information about general aspects of leprosy and an

explanation about our project and experimental procedures. We clinically evaluated and collected peripheral blood samples from those students who had the participation in the study consented by their responsible adult. When a new case was detected among the students, we went to the student's residence to evaluate their household contacts.

Data management and analysis

The spatial distribution pattern of leprosy cases in Castanhal was determined by combining information from the National Notifiable Diseases Information System (SINAN), the Brazilian Institute of Geography and Statistics (IBGE), and field-work mapping. The residences of people affected by leprosy in the urban area, reported during the last six years before our study, were georeferenced with a handheld GPS device to produce detailed maps of the leprosy distribution. Using a GIS (ArcGIS 10 - ESRI, Redlands, CA, USA), we draw point pattern maps, calculated the number of cases and the annual case detection rate per urban census tract and identified hyperendemic areas. Additionally, using the software Clusterseer 2.3 (Biomedware, Ann Arbor, MI, USA), we applied Kulldorff's spatial scan statistics [23] to identify clusters of leprosy. Detailed description of the spatial analysis we performed can be found in the Chapter 4 of this thesis. All examined SC also had their residential addresses georeferenced in order to analyze their spatial correlation with reported leprosy cases.

We used the Fisher's exact test to compare the proportion of new cases detected among seropositive and seronegative people or households. Mann-Whitney *U* tests was applied to compare the titers of anti-PGL-I IgM among different groups. The odds ratio of leprosy as a ratio of the probability of developing the disease and the number needed to harm (NNH) computed as 1/attributable risk, based on the seropositivity were also analyzed.

Results

Follow-up of individuals

From those 750 people initially evaluated in T1, we were able to reexamine 254 (33.8%, 94 males and 160 females), including 143 HHC and 111 SC, two years later (T2). Participants aged 5-80 years (mean = 20, SD = 14.1), 112 (44%) below 15 years old. The main reasons for non-participation in the follow-up were: (1) families that moved to unknown

addresses inside the same town (2), families that moved to other towns or states and (3) subjects that were out of home at the moment of our visit.

In T2, 43 (16.9%) people out of 254 were detected with leprosy. The incidence was significantly higher (p = 0.02) among those who tested positive to anti-PGL-I in than negative ones T1 (Table 2). The odds of developing overt leprosy in seropositive people were 2.7 times that of negative ones (p = 0.01, 95%CI = 1.29 – 5.87), pointing out that it is necessary to follow-up just 8 seropositive people to detect one case in two years (NNH). Figure 1 shows the progression of the antibody titration from T1 (no leprosy) to T2 (diagnosis). From those 43 new cases, 29 (67.4%) significantly increased (p = 0.001) the IgM titers (mean increase = 110%, SD = 80%; median titration in T1 = 0.333, IQR = 0.251; median in T2 = 0.686, IQR = 0.353). Otherwise, the decrease observed in the other 14 subjects was not statistically significant (mean decrease = 30%, SD = 20%; median titration in T1 = 0.956, IQR = 1.755; median in T2 = 0.723, IQR = 0.947; p = 0.278). During the first evaluation, 33 out of those 43 (76.7%) tested positive to anti-PGL-I; while in the diagnosis 39 (90.7%) were seropositive.

Table 2. Individuals evaluated twice in the cohort.

Serology (T1)[1]	Households visited	People examined	New cases detected in T2 (%)*	Paucibacillary	Multibacillary
Positive	113	148	33 (22.3%)	7	26
Negative	76	106	10 (9.4%)	2	8
Total	131#	254	43 (16.9%)	9	34

[1] T1 = First evaluation. T2 = second evaluation performed two years later.
* The difference is statistically significant (p = 0.027). Fisher's exact test.
# Most of the times there were positive and negative subjects in the same household.

The group that did not develop leprosy during this follow-up demonstrated significant increase in the average antibody titers, also (T1 – median OD = 0.336, IQR = 0.461; T2 – median OD = 0.460; IQR = 0.543). But, the most important increase in the IgM titers was observed in the group that developed the disease (T1 – median OD = 0.371, IQR = 0.359; T2 – median OD = 0.702, IQR = 0.562) (Figure 2). Despite that, 18/148 (12.1%) seropositive became negative during the study, while 60/106 (56.6%) seronegative became positive, including 7 that were detected with leprosy.

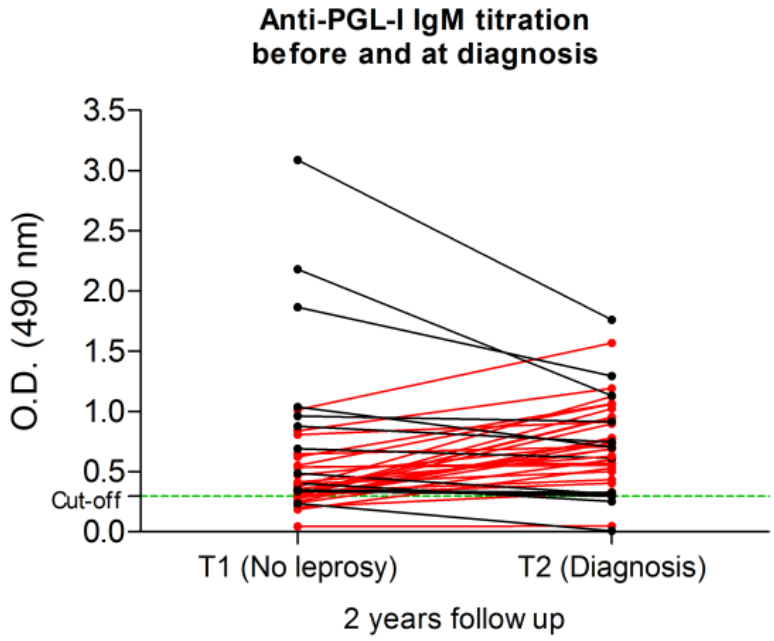

Figure 1. Anti-PGL-I IgM titration before and at diagnosis. The red lines/dots represents those people who increased the IgM titers (significant increase, p = 0.001), while black lines/dots means those who decreased the titration (not significant decrease, p = 0.278). All of them were detected with leprosy after 2 years follow-up.

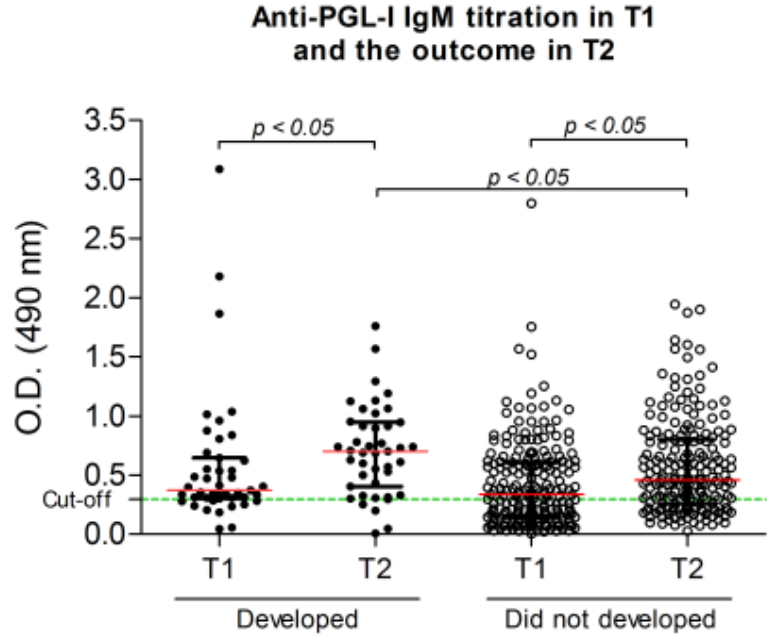

Figure 2. Anti-PGL-I IgM titration in the first (T1) and in the second (T2) evaluation. All HHC and SC evaluated twice (T1 and T2) are included in this figure. From 254 people, 43 (16.9%) developed overt disease and 211 did not in a period of 2 years follow-up. The most important increase in the IgM titers was observed in the group that developed the disease. Group "developed" (T1 – median OD = 0.371, IQR = 0.359; T2 – median OD = 0.702, IQR = 0.562). Group "did not developed" (T1 – median OD = 0.336, IQR = 0.461; T2 – median OD = 0.460; IQR = 0.543).

Positive versus negative houses

In T2, besides those 254 people evaluated twice, we also examined more 324 subjects that were not examined in T1, including both HHC of leprosy patients and HHC of seropositive or seronegative students. We generically classified households with at least one seropositive dweller as "positive houses" and, as "negative houses" those with just seronegative dwellers. Using this approach we detected additional 48 (14.8%) new cases, counting a total of 91. There were a significant difference (p = 0.028) in the incidence of new cases among people of "positive houses" compared to those of "negative houses". The odds of a new leprosy case in "positive houses" was 2.6 times that of negative ones (p = 0.02, 95%CI = 1.18 – 5.91), indicating that it is necessary to follow-up 10 people living in "positive houses" to detect one new case in a period of two years (NNH).

Table 3. Subjects evaluated in "positive or negative houses". [1]

Group (T1)[1]	Households visited	People examined	New cases detected in T2 (%)*	Paucibacillary	Multibacillary
"Positive house"	113	483	84 (17.4%)	27	57
"Negative house"	18	95	7 (7.4%)	2	5
Total	131	578	91 (15.7%)	29	62

[1] "Positive house" = households with at least one seropositive dweller. "Negative house" = household with just seronegative dwellers.
***** The difference is statistically significant (p = 0.02); Fisher's exact test. Odds ratio = 2.64 (p = 0.02, 95%CI = 1.18 – 5.91).

Survey of students in high risk areas

We also evaluated other 134 students, aged 6-14 years (mean = 10.4), of two elementary public schools placed in high risk areas of Castanhal. Eleven (8.2%) new leprosy cases were detected based on clinical signs and symptoms of the disease. Four were classified as PB leprosy and 7 as MB (4 BT and 3 BB). No physical disability was observed among these 11 cases; 4 (36.3%) reported previous contact with at least one leprosy case (household or close contacts) ranging from 3 to 5 years long and 3 (27.2%) had no BCG scar. The most frequent skin lesion was hypopigmented macules with loss of sensation.

A very high seroprevalence of anti-PGL-I IgM (77.6%) was observed in this sample (median OD of seropositive SC was 0.564; IQR = 0.296), but 5 out of 11 new cases (45.4%) tested negative, especially those PB and BT forms. There was no significant difference (p =

0.225) between the median OD of new cases (0.436; IQR = 0.287) compared to the median of healthy students (0.488; IQR = 0.337). We went to the residences of those SC newly detected with leprosy and examined 42 of their HHC, where other 7 (16.6%) new cases were diagnosed with leprosy. Twenty-three (54.7%) also tested positive to anti-PGL-I (median OD for those seropositive HHC was 0.657).

Analyzing the spatial distribution of leprosy cases reported during the period of 2004 to February 2010 with the location of the residences of the 134 evaluated SC (Figure 3), we observed that 22 (16.4%) were residing within 50 meters of at least one leprosy case; and 83 (62%) and 121 (90.3%) were dwelling less than 100 or 200 meters, respectively, from a known case. All the 11 new cases in SC were living within 200m, 6 (54.5%) and 1 (9.1%) within 100 or 50m, respectively, of at least one case. There was a significant difference ($p = 0.04$) in the proportion of new cases detected at the schools that were selected based on the spatial distribution of the reported cases (11 out of 134; 8.2%) when compared with our previous findings [3] in randomly selected schools (63 out of 1,592; 4%).

Overall clinical and epidemiological outcomes

Considering all 754 people included in this study, we detected a total of 109 (14.4%) new cases; 40 (36.7%) in children below 15 years old; 95 (87.2%) with DG 0 and 14 (12.8%) with DG 1; 64 (58.7%) were females; 91 (83.4%) had at least one BCG scar; 60 (55%) were living in crowded houses (more than 2 dwellers per bedroom); the average number of people per household was 5.4, but in 9 (9.8%) there were 10 or more dwellers; 17 (15.6%) reported an expectation to move to another place in the near future; 16 (14.7%) reported starvation at least one time in their lives; 55 (50.4%) had a family income of up to one Brazilian minimum wage per month (roughly 250 US dollars) and 77 (70.6%) have any kind of financial assistance from the federal government, especially the family or school allowance (Brazilian official income transfers programs).

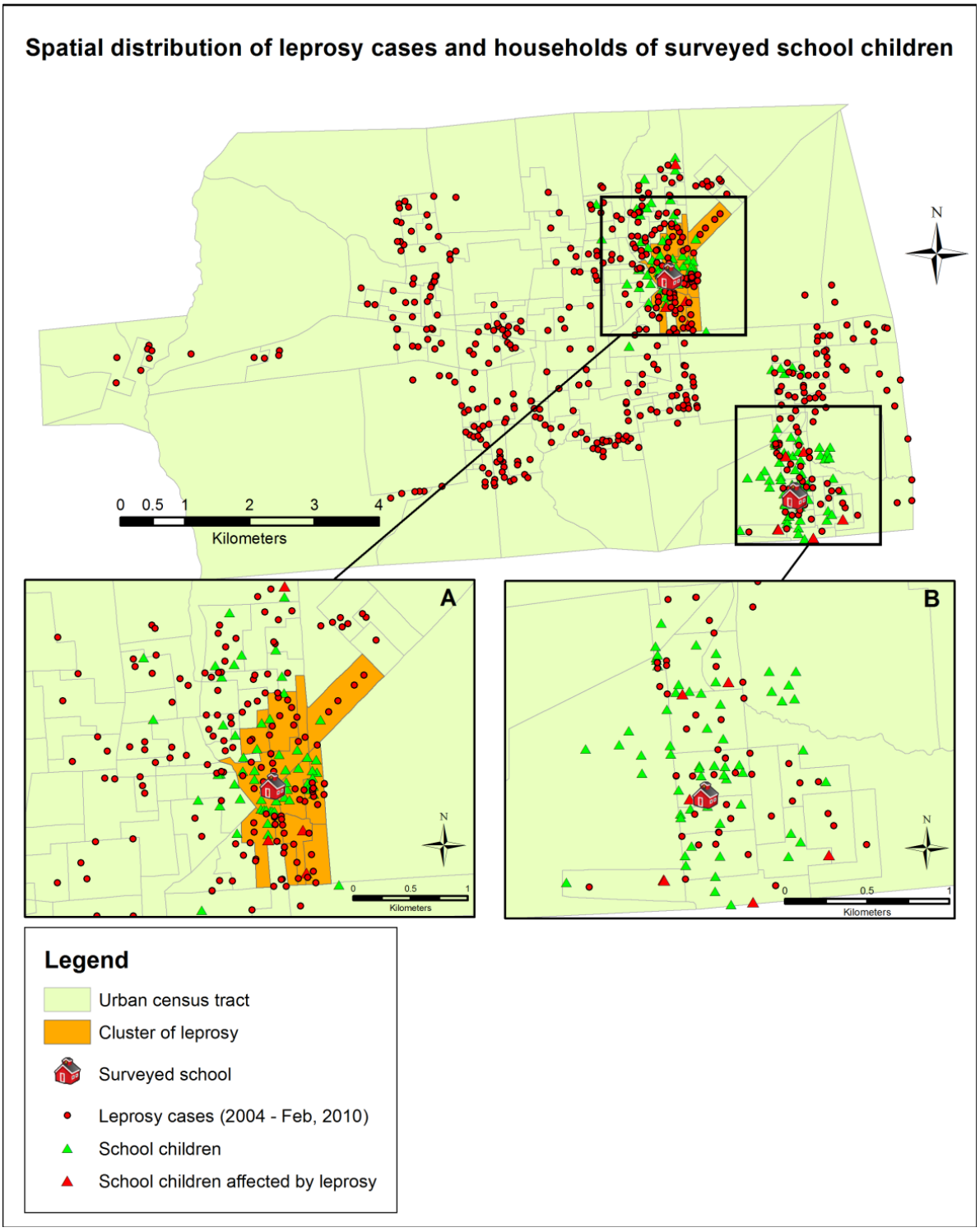

Figure 3. Spatial distribution of leprosy cases and household of surveyed school children. We mapped 499 (87%) reported cases in the urban area of Castanhal, detected from 2004 to February 2010, and the residences of 134 examined school children. We selected 2 schools placed in high risk areas: (A) The most likely cluster of leprosy cases determined by Kulldorff's spatial scan statistics (orange area); (B) A hyperendemic area in the periphery of the city. All 11 new cases detected among the SC were dwelling less than 200m of at least one known case.

Discussion

School based active clinical surveillance in high risk areas determined by spatial epidemiology, and targeted household contacts and families follow-up guided by serologic data significantly increases the early detection of new leprosy cases. Selecting schools located in a predefined cluster of leprosy or in a hyperendemic urban census tract of the city resulted in a two-fold higher detection rate when compared to our previous findings in randomly selected schools [3]. All new cases detected among SC were dwelling in close proximity to reported cases and this spatial correlation can also help to understand the extremely high prevalence of subclinical infection observed in this sample of students, once neighbors and extra-domiciliary contacts have an increased risk of leprosy too [24,25]. Serology to detect anti-PGL-I IgM demonstrated a significant capacity to be used as a biomarker of infection in an individual level as well as a landmark of households with an increased risk of leprosy. Fine scale spatial epidemiology and serologic data should be applied to increase the detection rate in hyperendemic regions of the globe.

The antibody titers has a moderate to good correlation with the bacterial load [26,27], and its responses against PGL-I and others protein antigens like LID-1 has been demonstrated to predict the onset of leprosy in armadillo model and clinical settings [8,28-31]. Our analysis indicates that 1 out of 8 seropositive people will progress to overt disease in a period of two years, and that the antibodies titers will significantly increase before the diagnosis in most of those who will develop the disease. However, seronegative HHC should not be neglected, especially in hyperendemic areas, because anti-PGL-I serology tests have poor sensibility (roughly 50%) even to detect those with established PB leprosy [32]. Moreover, in this study two years was an enough period of time to some seronegative individuals become positive and develop clinical manifestations of leprosy. We observed a slight, but statistically significant increase in the average antibody titers among those people that did not develop the disease during this follow-up. Since there are evidences that treating the index cases decreases the reactivity against *M. leprae* antigens in HHC, indicating that the continue exposure was broken [8], we suppose that the antibody titers will decline and eventually become negative in most of the HHC of those cases detected by our group.

Beyond to identify individual, serologic data was also able to identify household at most risk of leprosy. The probability of new cases in “seropositive houses” is more than two-fold higher as compared to “negative houses”. In a period of two years, 1 out of 10 people in

"positive houses" will progress to overt disease. Similar findings were obtained by a prospective study carried out in Cebu (Philippines), where HHC in approximately 1 of 7 households of MB leprosy patients developed leprosy during the 7-years period of active surveillance [33]. The authors suggested treating antibody-positive high risk household contacts, even with no clinical manifestation, with an MB leprosy treatment regimen to prevent transmission, but to our understanding, it seems unfeasible in settings with such extremely high seroprevalence of anti-PGL-I, like the State of Para. Some researchers have proposed chemoprophylaxis as an alternative strategy to interrupt the transmission of *M. leprae*, once it gives around 60% protection against the disease during the first 2 years [34-36], but it has not been widely recommended because still there are important doubts regarding the lasting of the protection, the development of new resistant strains, and its efficacy in such hyperendemic areas with high prevalence of undiagnosed cases.

If left untreated, leprosy can progress to irreversible physical disabilities, but it has been described that a significant number of individuals will experience mild signs and symptoms and they may never become registered cases if their leprosy heals spontaneously, as described in the literature [37-39]. According to Moet *et al.* [40], self-healing of leprosy could contributes to the difference between active and passive case-finding.

We just reevaluate 33.8% of those subjects surveyed in T1, what represent a limitation of this study. Moreover, more females than males were included because women frequently are in charge of domestic tasks and were at home at the moment of our visit, while men usually go out to work impacting on our sampling capacity. Considering that the international epidemiological data historically indicates a higher incidence of leprosy among males, we may have lost some cases during this study, underestimating the size of the problem. We classified a household as "negative house" based on those dwellers that we evaluated, but in some cases we were not able to examine all the residents, what can also be a source of bias, not detecting possible seropositive individuals in those "negative houses".

There are strong evidences that not just HHC, but also social contacts (at school, workplace, religious temples, etc.) and neighbors of leprosy cases are under increased risk of leprosy [24,25,41,42]. Based on that, it has been suggested that contact surveys should be not only focused on HHC but also extended to entire neighborhoods or villages and social contacts. However, in a regional scenario where less than 50% of HHC of reported leprosy cases were examined in the last 10 years, mainly because of the low coverage and inefficiency

of the local public health system in the State of Para, this is the old challenge: to evaluate all HHC of new leprosy cases and to extend contact tracing to a wider range of people at higher risk of leprosy, in a sustainable manner.

School children survey has been advocated as an important strategy for early detection since 1947 [43], but it is not a usual recommendation in the nationals and regionals control program despite of some evidence of its efficacy [44-48]. In an inedible manner, the 2013 Brazilian leprosy campaign concentrated its strategy on evaluating SC of public schools from highly endemic municipalities of the country. They screened 3.6 million students, using a self-evaluation scheme where the SC parents were in charge of pointing out suspicious skin lesions. Due to that, 238,000 SC were referenced to be clinically examined by a physician at basic healthy units and 283 (0.12%) were newly detected with leprosy (official data of the Ministry of Healthy, up to date in October 2013). It is a particularly alarming detection rate considering that it is among children, indicating active foci of infection in their communities, but based on our findings, we strongly believe that if large-scale SC survey would be performed in specific spatial clusters of leprosy in each municipality, the detection rate would be even higher and with a better cost-effectiveness.

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CAPÍTULO 6

CONCLUSÕES

Em síntese, as conclusões deste estudo são as seguintes:

1. A infecção subclínica pelo *M. leprae* está amplamente disseminada entre os contatos intradomiciliares de pessoas atingidas pela hanseníase, bem como entre os estudantes de escolas públicas do ensino fundamental e médio do Estado do Pará.

2. A alta prevalência de hanseníase e de infecção subclínica pelo *M. leprae* entre os escolares pode ser explicada pela existência de casos não diagnosticados entre seus contatos intradomiciliares e pela proximidade espacial de suas residências a dos casos registrados.

3. Os contatos intradomiciliares de pessoas atingidas pela hanseníase deveriam ser examinados periodicamente durante, no mínimo, o período médio de incubação da doença.

4. A sorologia anti-PGL-I mostrou-se capaz de identificar indivíduos e famílias com maior risco de hanseníase.

5. Um em cada oito indivíduos soropositivos para anti-PGL-I irá apresentar manifestações clínicas da hanseníase em um período de dois anos.

6. Um em cada dez moradores de residências com pelo menos um sujeito soropositivo para anti-PGL-I irá apresentar manifestações clínicas da hanseníase em um período de dois anos.

7. O seguimento de indivíduos com altos títulos de anticorpos anti-PGL-I, bem como de seus contatos intradomiciliares é uma estratégia eficaz e deveria ser adotada pelo programa de controle da hanseníase do Estado do Pará para aumentar a detecção precoce de casos novos, prevenir incapacidades físicas e quebrar a cadeia de transmissão da hanseníase.

8. Há heterogeneidade na distribuição espaço-temporal da hanseníase na região urbana do município de Castanhal, com formação de *clusters* de alta e baixa taxa de detecção de casos em regiões específicas da cidade.

9. Estudantes soropositivos para anticorpo anti-PGL-I residem nas proximidades de *clusters* espaço-temporais de casos de hanseníase.

10. A avaliação clínica dermatoneurológica de estudantes da rede pública de ensino fundamental e médio no Estado do Pará é uma estratégia eficaz para aumentar a detecção precoce de casos novos de hanseníase, e deveria ser adotada permanentemente pelo programa de controle da endemia no Estado.

11. A seleção das escolas públicas a serem incluídas em programas de combate à hanseníase, com base em informações sobre a epidemiologia espacial da doença na escala dos setores censitários, aumenta significativamente a eficiência da estratégia de avaliação clínica dos estudantes para a detecção precoce de novos casos.

Com base na proporção de casos novos detectados na amostra avaliada, a prevalência não diagnosticada de hanseníase entre os contatos intradomiciliares de pessoas atingidas pela doença nos últimos seis anos no Pará é estimada em 802/10.000 contatos. Entre os estudantes de escolas públicas do ensino fundamental e médio do Pará a estimativa é de 396/10.000, indicando que atualmente existam aproximadamente 80.000 casos sem diagnóstico nesta população específica.

Deste modo, para que a meta de controle da hanseníase (prevalência < 1/10.000 habitantes) seja alcançada no Pará, é necessário identificar e tratar adequadamente os casos ocultos no Estado. Caso não haja um significativo aumento na detecção de casos novos, a hanseníase permanecerá como um problema de saúde pública no Pará durante as próximas décadas.

A contribuição da epidemiologia espacial no aumento da detecção de casos novos deve ser avaliada em municípios com diferentes características demográficas, como em pequenos municípios do interior e na capital. Novos estudos são necessários para determinar um cut-off sorológico sensível e específico o suficiente para predizer a ocorrência da hanseníase e permitir o tratamento do indivíduo antes mesmo do surgimento das manifestações clínicas. A relação custo-benefício das ferramentas e estratégias apresentadas nesta tese e a sua aplicabilidade na rede de assistência à saúde disponível no Pará ainda precisam ser avaliadas.

Curriculum vitae

Nascido em Castanhal (PA) em 7 de maio de 1976, filho de retirantes baianos, fui para Belém em 1994, ainda aos 17 anos de idade, para cursar a faculdade de fisioterapia na Universidade do Estado do Pará (UEPA). Após a graduação, imediatamente segui para São Paulo, onde me especializei em reabilitação reumatológica pela Universidade Federal de São Paulo, sendo bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo. Retornando a Castanhal, exerci a fisioterapia clínica em serviços privados e públicos, incluindo o cargo de fisioterapeuta na antiga colônia de hansenianos da Vila do Prata, em Igarapé-Açu (PA). Fui professor substituto das disciplinas de cinesiologia e cinesioterapia nos cursos de educação física e fisioterapia da UEPA por três semestres (2003-2004). Esta experiência foi importante para reforçar o antigo sonho da docência superior. Em 2005 fui aprovado em concurso público para o cargo de professor efetivo do Campus Universitário de Castanhal da Universidade Federal do Pará (UFPA), onde ministro, entre outras, a disciplina de saúde coletiva na Faculdade de Educação Física. Ingressei no ano seguinte, como aluno de mestrado, no Programa de Pós-graduação em Doenças Tropicais da UFPA, sob orientação do Prof. Claudio Guedes Salgado. Neste período (2006-2008) investiguei os efeitos da laserterapia de baixa intensidade sobre a cicatrização de úlceras hansênicas, uma sequela da doença que me angustiava desde os tempos do trabalho na antiga Colônia do Prata. O estudo foi realizado na Unidade de Referência Especializada em Dermatologia Sanitária Dr. Marcello Candia, em Marituba (PA), e os resultados apresentados no *17th International Leprosy Congress* (Hyderabad, India, 2008) e publicados na *BMC Infectious Diseases* em 2010 (doi: 10.1186/1471-2334-10-237). Em 2009, um ano após a conclusão do mestrado, retornei ao laboratório do Prof. Claudio Salgado (Laboratório de Dermato-Imunolgia) propondo um projeto de doutorado ainda em hanseníase, mas com um enfoque completamente diferente daquele da dissertação: agora eu queria prevenir as úlceras ajudando a diagnosticar precocemente a hanseníase. Nos últimos quatro anos, além dos artigos e manuscritos contidos nesta tese, fui coautor de outras três publicações: **[1]** *N Engl J Med.* 2012 Apr 12; 366(15): 1433. **[2]** *Emerg Infect Dis.* 2012 May; 18(5): 889-90. **[3]** *Acta Derm Venereol.* 2012 May; 92(3): 335. Fui bolsista da CAPES dentro do programa Ciência sem Fronteiras, realizando seis meses de estágio de doutorado sanduiche no exterior, no Departamento de Estudos Ambientais da *Emory University* (Atlanta, GA, USA) sob orientação do Prof. Uriel Kitron, onde recebi treinamento em sistemas de informação geográfica, análise estatística espacial e epidemiologia espacial. Também colaborei nas dissertações de mestrado de dois estudantes, orientei um trabalho de conclusão de curso de graduação e quatro alunos de ensino médio, bolsistas do Programa de Bolsas de Iniciação Científica Junior (PIBICJr - Fundação Amazônia Paraense de Amparo à Pesquisa); os cinco últimos envolvendo georreferenciamento de casos de hanseníase. Recentemente, em função do meu trabalho com epidemiologia espacial e sorológica da hanseníase no Estado do Pará, recebi o prêmio de jovem cientista no *18th International Leprosy Congress*, realizado em Bruxelas, Bélgica (2013), pela melhor apresentação oral no tema epidemiologia e controle.

ANEXOS E APÊNDICES

ANEXO 1 – Parecer do Comitê de Ética em Pesquisa Envolvendo Seres Humanos.

Universidade Federal de Pará

COMITÊ DE ÉTICA EM PESQUISA EM SERES HUMANOS DO INSTITUTO DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DO PARÁ

Carta: 06/08 CEP-ICS/UFPA

Belém, 21 de fevereiro de 2008.

Ao:
Prof. Dr. .Cláudio Guedes Salgado

Senhor Pesquisador,

Temos a satisfação de informar que seu projeto de pesquisa **"Detecção e análise da variação genotípica do mycobacterium leprae de casos-índice e comunicantes em regiões endêmicas do Estado do Pará"** de Protocolo **nº197/07, CEP-ICS/UFPA**, foi apreciado e aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Ciências da Saúde da Universidade Federal do Pará, na reunião do dia 04 de dezembro de 2007.

Assim, Vossa Senhoria tem o compromisso de entregar o relatório parcial do mesmo até o dia 30 de dezembro de 2008, no CEP-ICS/UFPA, situado no Campus Universitário do Guamá, Campus profissional, no Complexo de sala de aula do ICS – sala 13 (Altos).

Atenciosamente,

Profª. *M. Sc.* Maria da Conceição S. Fernandes.
Coordenadora do CEP-ICS/UFPA

Comitê de Ética em Pesquisa em Seres Humanos do Instituto de Ciências da Saúde da Universidade Federal do Pará (CEP-ICS/UFPA) - Complexo de Sala de Aula/ CCS - Sala 13 - Campus Universitário do Guamá, nº 01, Guamá – CEP: 66075-110 - Belém-Pará. Tel./Fax. 3201-8028/3201-7735 E-mail: cepccs@ufpa.br

APÊNDICE 1 – Questionário e ficha de avaliação dos casos-índice e comunicantes (páginas 128 a 147).

Detecção e Análise da Variação Genotípica do *Mycobacterium leprae* de Casos-índice e de Comunicantes, em Regiões Endêmicas do Estado do Pará.

486256/2007-3

Este projeto tem a participação das seguintes instituições:

Universidade Federal do Pará
Instituição Coordenadora

Laboratório de Dermato-Imunologia

Unidade de Referência Especializada Dr. Marcelo Cândia

Conselho Nacional de Desenvolvimento Científico e Tecnológico

Número de série: 0999

ÍNDICE

LABORATÓRIO DE DERMATO-IMUNOLOGIA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO DO PACIENTE

UNIVERSIDADE FEDERAL DO PARÁ - URE "MARCELLO CANDIA".

Eu _____,

Doc: _____, fui convidado(a) a participar do estudo "**Detecção e análise da variação genotípica do *Mycobacterium leprae* de casos-índice e comunicantes em regiões endêmicas do Estado do Pará**" e recebi do seu pesquisador responsável, informações que me fizeram entender, sem dificuldades ou dúvidas, os seguintes aspectos:

O estudo tem por objetivo, identificar características relacionadas às pessoas, aos seus modos de vida, ao meio ambiente e à genética da bactéria que causa a hanseníase, buscando entender mais sobre a transmissão da doença e melhorando sua prevenção e seu tratamento.

Os participantes deste estudo responderão a um questionário, serão submetidos à biópsia de pele, à coleta de sangue e de secreção nasal, realizadas por médicos e profissionais de saúde experientes, sempre com a finalidade de diagnosticar a doença. Suas casas serão visitadas, para que seja realizado o mapeamento da doença por ruas e bairros do município. As pessoas de convívio mais próximo aos participantes, também serão examinadas e submetidas à coleta de sangue e de secreção nasal de forma rápida e sem dor, desde que devidamente autorizado, bem como, serão orientadas sobre os sinais e sintomas da hanseníase.

A pesquisa será realizada em ambiente seguro, com material limpo e descartável, utilizando medicamentos confiáveis, oferecidos gratuitamente pelo sistema único de saúde e administrados por um longo período, com acompanhamento e atenção a qualquer complicação pela unidade de saúde.

A coleta de sangue poderá causar dor e pequena reação no local e a biópsia cutânea um pequeno ferimento na pele.

Sempre que desejar, serão fornecidos esclarecimentos sobre todas as etapas do estudo e a qualquer momento, o participante poderá recusar-se a continuar no estudo e retirar seu consentimento, sem que isso lhe traga qualquer penalidade ou prejuízo.

Está garantido o cumprimento do que fora informado acima, a gratuidade de todos os procedimentos e o sigilo da identidade dos participantes.

Finalmente, tendo compreendido todas as informações sobre a minha participação no estudo e estando consciente dos meus direitos, das minhas responsabilidades, dos riscos e dos benefícios que a minha participação implica, concordo em participar deste estudo e, para isso, DOU O MEU CONSENTIMENTO.

_____

Assinatura ou impressão dactiloscópica do(a) voluntário(a) ou responsável legal.

Local: _____ Data: _____/_____/_____

Prof. Dr. Claudio Guedes Salgado
CRM-PA 5062
Laboratório de Dermato-Imunologia
Av. João Paulo II, 113. Marituba, Pará, Brasil. Fone: (91) 3256-9097

LABORATÓRIO DE DERMATO-IMUNOLOGIA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO DO COMUNICANTE

0999 A

UNIVERSIDADE FEDERAL DO PARÁ - URE "MARCELLO CANDIA".

Eu, _____,
Doc: _____ , fui convidado(a) a participar do estudo "**Detecção e análise da variação genotípica do *Mycobacterium leprae* de casos-índice e comunicantes em regiões endêmicas do Estado do Pará**" e recebi do seu pesquisador responsável, informações que me fizeram entender, sem dificuldades ou dúvidas, os seguintes aspectos:

O estudo tem por objetivo, identificar características relacionadas às pessoas, aos seus modos de vida, ao meio ambiente e à genética da bactéria que causa a hanseníase, buscando entender mais sobre a transmissão da doença e melhorando a prevenção e o tratamento.

Os participantes deste estudo responderão a um questionário, serão submetidos à coleta de sangue e de secreção nasal, realizadas por médicos e profissionais de saúde experientes, sempre com a finalidade de diagnosticar a doença. Suas casas serão visitadas, para que seja feito um mapeamento da doença por ruas e bairros do município.

A pesquisa será realizada em ambiente seguro, com material limpo e descartável. A coleta de sangue poderá causar dor e pequena reação no local e a coleta de secreção nasal será realizada de forma rápida e indolor.

A participação nesta pesquisa é voluntária e, sempre que desejar, serão fornecidos esclarecimentos sobre todas as etapas do estudo. A qualquer momento, o participante poderá recusar-se a continuar no estudo e retirar seu consentimento, sem que isso lhe traga qualquer penalidade ou prejuízo.

Está garantido o cumprimento do que fora informado acima, a gratuidade de todos os procedimentos e o sigilo da identidade dos participantes.

Finalmente, tendo compreendido todas as informações sobre a minha participação no estudo e estando consciente dos meus direitos, das minhas responsabilidades, dos riscos e dos benefícios que a minha participação implica, concordo em participar deste estudo e, para isso, DOU O MEU CONSENTIMENTO.

_____

Assinatura ou impressão dactiloscópica do(a) voluntário(a) ou responsável legal.

Local:_____ Data: _____/_____/_____

Prof. Dr. Claudio Guedes Salgado
CRM-PA 5062
Laboratório de Dermato-Imunologia
Av. João Paulo II, 113. Marituba, Pará, Brasil. Fone: (91) 3256-9097

LABORATÓRIO DE DERMATO-IMUNOLOGIA

INFORMAÇÕES PESSOAIS

1 **Nome:** _____

2 **Nascimento:** _____/_____/_____ 3 **Idade:** _____ **anos** 4 **Gênero:** ☐ M ☐ F

5 **Naturalidade (Cidade/UF):** _____

6 **Estado Civil:** ☐ Solteiro ☐ Casado(a) ☐ União estável ☐ Separado(a) ☐ Viúvo(a)

7 **Cor da Pele/Etnia:** ☐ Negra ☐ Branca ☐ Parda ☐ Amarela ☐ Indígena

8 **Escolaridade (em anos de estudos concluídos):**

☐ 1 a 3 ☐ 4 a 7 ☐ 8 a 12 ☐ 13 ou mais ☐ Não se aplica

Maior grau de escolaridade atingido:

☐ Nenhum ☐ Ensino Fundamental ☐ Ensino Médio ☐ Ensino Superior

9 **Ocupação:** _____

☐ Em exercício ☐ Afastado Temporariamente ☐ Desocupado

10 **Renda do núcleo familiar:**

☐ Sem renda ☐ < 1 Salário mínimo ☐ 1 Salário mínimo

☐ Até 2 salários mínimos ☐ Até 3 salários mínimos ☐ > 3 Salários mínimos

11 **O núcleo familiar é beneficiado com algum tipo de transferência governamental?**

☐ Não

☐ Sim. Qual? ☐ Aposentadoria ☐ Pensão permanente ☐ Pensão temporária

☐ Programa oficial de auxílio: _____

☐ Outro: _____

12 **Já sofreu privação alimentar?**

☐ Sim ☐ Não

LABORATÓRIO DE DERMATO-IMUNOLOGIA

INFORMAÇÕES DOMICILARES

13 **Endereço:** _____

Ponto de referência: _____

14 **Bairro/Cidade/UF:** _____ 15 **CEP:** _____ - _____

16 **Zona:** ☐ Urbana ☐ Rural ☐ Urbana/Rural ☐ Ignorado

17 **Fones:**(___) _____ Cel: (___) _____ Contato: (___) _____

18 **Georreferencimento do domicílio**

☐ Não ☐ Sim Latitude: _____°_____'_____" Longitude: _____°_____'_____"

19 **Tempo de residência no domicílio:** _____

20 **Número de cômodos na casa:** _____ 21 **Total de moradores:** _____ Pessoas

22 **Há dormitório(s) com densidade acima de 2 pessoas?** ☐ Sim ☐ Não

23 **Residências anteriores:**

(Bairro/Cidade/UF): _____ Por quanto tempo? _____

(Bairro/Cidade/UF): _____ Por quanto tempo? _____

24 **Expectativa de mudança?** ☐ Não ☐ Sim. Bairro/Cidade/UF: _____

25 **Abastecimento de água:**

☐ Rede de água encanada ☐ Poço ''boca larga''

☐ Poço artesiano ☐ Outro: _____

26 Água para consumo: ☐ Mineral ☐ Filtrada, fervida ou clorada ☐ Coada ou nenhum outro método

27 Rede de esgoto: ☐ Pública ☐ Fossa ☐ Outro: _____

28 Destino do lixo: ☐ Coleta ☐ Queima ☐ Enterra ☐ A céu aberto

29 Domicílio subnormal? ☐ Sim ☐ Não

08

LABORATÓRIO DE DERMATO-IMUNOLOGIA

CONTATO COM HANSENÍASE

30 **Conviveu com caso(s) de hanseníase, previamente?**

☐ Não ☐ Sim Quantos? ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ou mais

30.1 Nome: _____

Grau de Parentesco: ☐ Pais ☐ Namorado(a) ☐ Parente não Consanguineo
☐ Irmãos ☐ Cônjuge ☐ Não Parente
☐ Filho(a) ☐ Parente Consanguineo

Tipo de convívio: ☐ Intra-domiciliar ☐ Extra-domiciliar

Período de convivência: ☐ < 1 ☐ 1 a 2 ☐ 2 a 5 ☐ 5 a 10 ☐ > 10 anos

Há mais de 10 anos? ☐ Sim ☐ Não

30.2 Nome: _____

Grau de Parentesco: ☐ Pais ☐ Namorado(a) ☐ Parente não Consanguineo
☐ Irmãos ☐ Cônjuge ☐ Não Parente
☐ Filho(a) ☐ Parente Consanguineo

Tipo de convívio: ☐ Intra-domiciliar ☐ Extra-domiciliar

Período de convivência: ☐ < 1 ☐ 1 a 2 ☐ 2 a 5 ☐ 5 a 10 ☐ > 10 anos

Há mais de 10 anos? ☐ Sim ☐ Não

30.3 Nome: _____

Grau de Parentesco: ☐ Pais ☐ Namorado(a) ☐ Parente não Consanguineo
☐ Irmãos ☐ Cônjuge ☐ Não Parente
☐ Filho(a) ☐ Parente Consanguineo

Tipo de convívio: ☐ Intra-domiciliar ☐ Extra-domiciliar

Período de convivência: ☐ < 1 ☐ 1 a 2 ☐ 2 a 5 ☐ 5 a 10 ☐ > 10 anos

Há mais de 10 anos? ☐ Sim ☐ Não

LABORATÓRIO DE DERMATO-IMUNOLOGIA

DIAGNÓSTICO

31 **Data do diagnóstico:** ____/____/____ 32 **Unidade de Saúde:** _____

33 **Prontuário:** _____ 34 **SINAN:** _____

35 **Recidiva:** ☐ Não ☐ Sim

36 **Foto:** ☐ Não ☐ Sim. Registro: _____

37 **Grau de Incapacidade física no diagnóstico:**

☐ 0 ☐ 1 ☐ 2 ☐ Não realizado

38 **Cicatriz BCG:**

Número de cicatrizes: ☐ 0 ☐ 1 ☐ 2 ☐ Duvidosa

Diâmetro das cicatrizes _____ mm; _____ mm.

39 **Tipos de leões:**

S - Somente área hipoestésica

M - Mácula hipocrômica

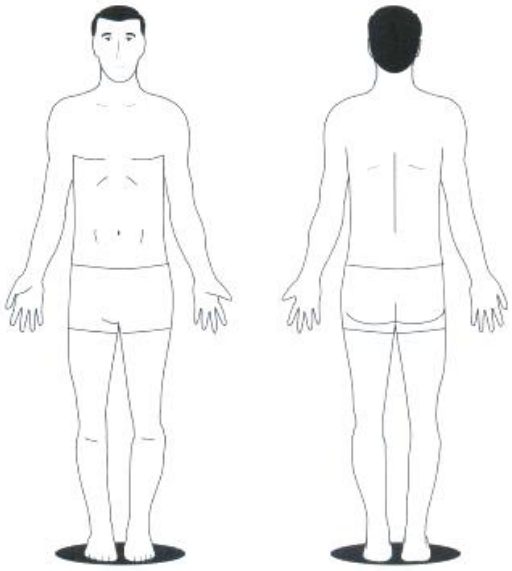
T - Tubérculo

P - Placa

F - Fóvea

I - Infiltração

N - Nódulo



40 **Número de lesões:** ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ou mais

LABORATÓRIO DE DERMATO-IMUNOLOGIA

DIAGNÓSTICO

41 **Forma clínica:**

☐ I ☐ T ☐ BT ☐ BB ☐ BV ☐ V ☐ Neural pura

42 **Classificação operacional:** ☐ PB ☐ MB

43 **Co-morbidades:**

Diabetes Melitus ☐ Sim ☐ Não

Hipertensão Arterial Sistêmica ☐ Sim ☐ Não

Neoplasias ☐ Sim ☐ Não

Outras: _____

_____

44 **Co-infecções:**

HIV ☐ Sim ☐ Não

Tuberculose ☐ Sim ☐ Não

Hepatite C ☐ Sim ☐ Não

Outras: _____

_____

LABORATÓRIO DE DERMATO-IMUNOLOGIA

LABORATÓRIO

45 **Baciloscopia:** ☐ Positiva ☐ Negativa ☐ Não realizada

IB: _____

IM: _____

Data e local do teste: ____/____/____ , _____

Técnico responsável: _____

46 **Histopatologia:** ☐ I ☐ T ☐ BT ☐ BB ☐ BV ☐ V ☐ Não realizada

47 **Sorologia:**

ELISA (Anti-PGL1): _____ DO: _____ *Cut-off:* _____

Data e local do teste: ____/____/____, _____

Técnico responsável: _____

☐ Não realizada

48 **Diagnóstico molecular:**

☐ Positivo ☐ Negativo ☐ Não realizado

49 **Sensibilidade medicamentosa - Sequenciamento:**

rpoB mutação ☐ Não ☐ Sim ☐ Não realizado

folP mutação ☐ Não ☐ Sim ☐ Não realizado

gyrA mutação ☐ Não ☐ Sim ☐ Não realizado

50 **Genotipagem:**

_____

_____

☐ Não realizado

LABORATÓRIO DE DERMATO-IMUNOLOGIA

TRATAMENTO

51 **Esquema de tratamento:**

- ☐ PQT/PB 6 doses
- ☐ PQT/MB 12 doses
- ☐ PQT/MB 24 doses
- ☐ Esquema alternativo: _____

52 **Reação hansênica antes do tratamento:**

- ☐ Não
- ☐ Sim

Tipo de reação:

☐ 1 ☐ 2 ☐ Mista ☐ Neurite isolada

Tratamento:

☐ Prednisona ☐ Talidomida ☐ Outro: _____

LABORATÓRIO DE DERMATO-IMUNOLOGIA

RECIDIVA

* Procedência das informações: ☐ Prontuário ☐ Paciente ☐ Acompanhante

53 **Data do 1º diagnóstico:** _____/_____/_____

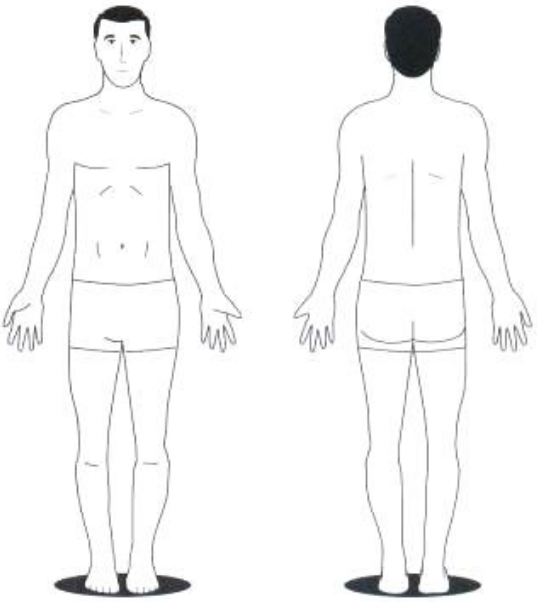
54 **Quantos episódios de recidiva o paciente já apresentou?** _____

55 **Local do 1º diagnóstico:** _____

☐ Mesma US ☐ Outra US ☐ US Referência

56 **Tipos de lesões observadas no 1º diagnóstico**

S - Somente área hipoestésica
M - Mácula hipocrômica
T - Tubérculo
P - Placa
F - Fóvea
I - Infiltração
N - Nódulo



57 **Número de lesões:**

☐ 1 ☐ 2 ☐ 3
☐ 4 ☐ 5 ☐ 6 ou mais

58 **As lesões observadas no 1º diagnóstico desapareceram após o tratamento?**

☐ Sim ☐ Não

59 **Forma clínica no 1º diagnóstico:**

☐ I ☐ T ☐ BT ☐ BB ☐ BV ☐ V ☐ Neural pura ☐ Não sabe informar

60 **Classificação operacional:** ☐ PB ☐ MB

61 **Esquema terapêutico após o 1º diagnóstico:**

☐ PQT/PB 6 doses ☐ PQT/MB 12 doses ☐ PQT/PB 24 doses
☐ DNDS + PQT ☐ ROM ☐ Esquema alternativo: _____

LABORATÓRIO DE DERMATO-IMUNOLOGIA

RECIDIVA

62 **Grau de Incapacidade física no 1º diagnóstico:**

☐ 0 ☐ 1 ☐ 2 ☐ Não realizado ☐ Não sabe informar

63 **Apresentou reação durante o 1º tratamento?** ☐ Sim ☐ Não

Tipo de reação: ☐ Tipo 1 ☐ Tipo 2 ☐ Mista ☐ Neurite isolada

Tratamento: ☐ Prednisona ☐ Talidomida ☐ Outros

Resposta: ☐ Boa ☐ Ruim

64 **Nº de trocos nervosos afetados no 1º diagnóstico:**

☐ 0 ☐ 1 ☐ 2 ou mais

☐ Radial direito ☐ Radial esquerdo

☐ Ulnar direito ☐ Ulnar esquerdo

☐ Mediano direito ☐ Mediano esquerdo

☐ Fibular direito ☐ Fibular esquerdo

☐ Tibial direito ☐ Tibial esquerdo

☐ Outros _____

☐ Não sabe informar

65 **Baciloscopia no 1º diagnóstico:**

☐ Positiva ☐ Negativa ☐ Não realizada ☐ Não sabe informar

IB: _____

IM: _____

LABORATÓRIO DE DERMATO-IMUNOLOGIA

RECIDIVA

66 **Histopatologia no 1º diagnóstico:**

☐ I ☐ T ☐ BT ☐ BB ☐ BV ☐ V ☐ Não realizada

67 **Apresentou reação hansênica no período pós-alta?**

☐ Sim ☐ Não

Tipo de reação: ☐ Tipo 1 ☐ Tipo 2 ☐ Mista ☐ Neurite isolada ☐ Não sabe informar

Quanto tempo após a alta?

☐ Menos de 3 meses ☐ De 3 a 12 meses ☐ Mais de 12 meses

Quantos episódios reacionais?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Mais de 5

Esquema de Tratamento:

☐ Prednisona. Período: _____ meses

☐ Talidomida. Período: _____ meses

☐ P + T. Período: _____ meses

☐ Outro: _____ Período _____ meses

Tratamento Continuo: ☐ Sim ☐ Não

Resposta: ☐ Boa ☐ Ruim

68 **Apresentou outra doença durante o período pós-alta?**

☐ Não

☐ Sim, Qual? _____

LABORATÓRIO DE DERMATO-IMUNOLOGIA

COMUNICANTE

69 **Comunicantes do paciente:** A

0999

69.1 **Nome:** _____

69.1.1 **Grau de Parentesco:**

☐ Pais ☐ Namorado(a) ☐ Parente não Consanguineo

☐ Irmãos ☐ Cônjuge ☐ Não Parente

☐ Filho(a) ☐ Parente Consanguineo

69.1.2 **Período de convivência:** _____

69.1.3 **Tipo de convívio:** ☐ Intra-domiciliar ☐ Extra-domiciliar

Telefone: (___)_____ ; (___)_____ ; (___)_____

Endereço: _____

Ponto de referência: _____

Georeferenciamento do domicílio:

☐ Não ☐ Sim Latitude: ____°____'____" Longitude: ____°____'____"

69.1.4 **Cicatriz BCG:**

Número de cicatrizes: ☐ 0 ☐ 1 ☐ 2 ☐ Duvidosa

Diâmetro das cicatrizes _____ mm; _____ mm.

69.1.5 **Forma clínica:** ☐ I ☐ T ☐ BT ☐ BB ☐ BV ☐ V ☐ Neural pura

69.1.6 **Classificação Operacional:** ☐ PB ☐ MB

69.1.7 **Presença de lesões:** ☐ Sim ☐ Não

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69.1.8 Tipos de lesões:

S - Somente área hipoestésica

M - Mácula hipocrômica

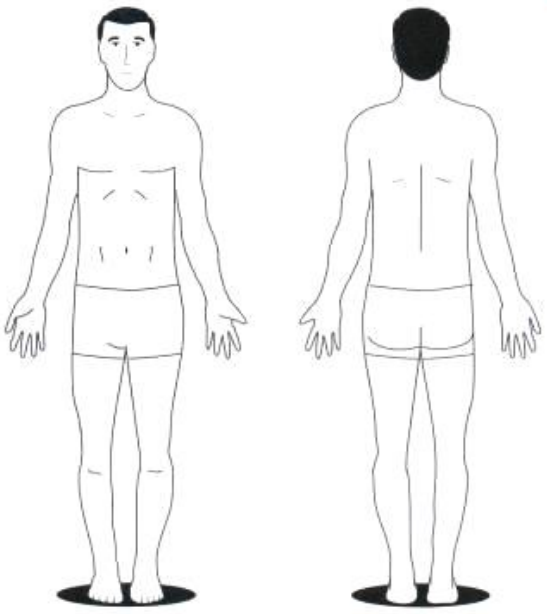
T - Tubérculo

P - Placa

F - Fóvea

I - Infiltração

N - Nódulo



Número de lesões:

☐ 1 ☐ 2 ☐ 3

☐ 4 ☐ 5 ☐ 6 ou mais

69.1.9 Sorologia:

ELISA (Anti-PGL1): _____ DO: _____ *Cut-off:* _____

Data e local do teste: ____/____/____, _____

Técnico responsável: _____

69.1.10 Diagnóstico molecular:

☐ Positivo ☐ Negativo ☐ Não realizado

69.1.11 Comunicante foi diagnosticado como caso novo?

☐ Não ☐ Sim. Ver questionário, número de série: _____

Observações:

_____

_____

_____

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AVALIAÇÃO NEUROLÓGICA SIMPLIFICADA

FACE	1• / /		2• / /		3• / /	
Nariz	D	E	D	E	D	E
Queixa principal						
Ressecamento (S/N)						
Ferida (S/N)						
Perfuração de septo (S/N)						
Olhos	D	E	D	E	D	E
Queixa principal						
Fecha olhos s/ força (mm)						
Fecha olhos c/ força (mm)						
Triquíase (S/N) / Ectrópio (S/N)						
Dimin. sensib. córnea (S/N)						
Opacidade da córnea (S/N)						
Catarata (S/N)						
Acuidade visual						

Membros superiores	1• / /		2• / /		3• / /	
Queixa principal						
Palpação de nervos	D	E	D	E	D	E
Ulnar						
Mediano						
Radial						

Legenda: N = normal E = espessado D = dor

Avaliação da força	1• / /		2• / /		3• / /	
	D	E	D	E	D	E
Abrir dedo mínimo Abdução do 5º dedo (nervo ulnar)						
Elevar o polegar Abdução do polegar (nervo mediano)						
Elevar o polegar Extensão do punho (nervo radial)						

Legenda: F = Forte D = Dimiunuida P = Paralisado ou 5 = Forte, 4 = Resistência Parcial, 3 = Movimento completo, 2 = Movimento parcial, 1 = Contração, 0 = Paralisado

Inspeção e avaliação sensitiva

1• / /		2• / /		3• / /	
D	E	D	E	D	E

Legenda: Caneta/filamento lilás (2 g): Sente ✓ Não sente X ou Monofilamentos: seguir cores

Garra móvel: M Garra rígida: R Reabsorvição: Ferida:

LABORATÓRIO DE DERMATO-IMUNOLOGIA

AVALIAÇÃO NEUROLÓGICA SIMPLIFICADA

Membros superiores	1• / /		2• / /		3• / /	
Queixa principal						
Palpação de nervos	**D**	**E**	**D**	**E**	**D**	**E**
Fibular						
Tibial Posterior						

Legenda: N = normal E = espessado D = dor

Avaliação da força	1• / /		2• / /		3• / /	
	D	**E**	**D**	**E**	**D**	**E**
Elevar o hálux Extensão de hálux (nervo fibular) 						
Elevar o pé Dorsiflexão de pé (nervo fibular) 						

Legenda: F = Forte D = Dimiunuida P = Paralisado ou 5 = Forte, 4 = Resistência Parcial, 3 = Movimento completo, 2 = Movimento parcial, 1 = Contração, 0 = Paralisado

Inspeção e avaliação sensitiva

1• / /		2• / /		3• / /	
D	**E**	**D**	**E**	**D**	**E**
					

Legenda: Caneta/filamento lilás (2 g): Sente  Não sente X ou Monofilamentos: seguir cores

Garra móvel: M Garra rígida: R Reabsorção:  Ferida: 

CLASSIFICAÇÃO DO GRAU DE INCAPACIDADE (OMS)

DATA DA AVALIAÇÃO	OLHOS		MÃOS		PÉS		MAIOR GRAU		ASSINATURA
	D	E	D	E	D	E	D	E	
Aval. diagnóstico / /									
Aval. diagnóstico / /									

LEGENDA PARA PREENCHMENTO DO GRAU DE INCAPACIDADE

GRAU	CARACTERÍSTICAS
0	Nenhum problema com os olhos, mãos e pés decorrente da hanseníase
I	Diminuição ou perda da sensabilidade nos olhos Diminuição ou perda da sensabilidade nas mãos e/ou pés (não sente 2 g ou toque da caneta)
II	Olhos: lagoftalmo e/ou ectrópio; triquíase; opacidade corneana central; acuidade visual menor que 0, 1 ou não conta dedos a 6 m. Mãos: lesões tróficas e/ou traumáticas; garras; reabsorção; pé caído; contratura do tornozelo.

MONOFILAMENTOS

COR	Gramas
Verde	0,05
Azul	0,2
Lilás	2,0
Verm. fechado	4,0
Verm. cruzado	10,0
Verm. aberto	300,0
Preto	s/resposta

INSTRUÇÕES PARA PREENCHIMENTO

1. Preencher com nome completo do paciente, sem abreviações.
2. Preencher com a data de nascimento do paciente (dia/mês/ano) de forma completa.
3. Anotar a idade do paciente.
4. Assinalar a categoria referente ao sexo do paciente.
5. Preencher com o nome completo do município e com a sigla do Estado onde o paciente nasceu.
6. Assinalar a categoria referente ao estado civil do paciente.
7. Assinalar a cor da pele/etnia auto-declarada pelo paciente.
8. Assinalar o número de anos de estudo concluídos pelo paciente. Considerar cada série concluída com aprovação como um ano de estudo. (Ex.: O paciente cursou 4 anos, porém não concluiu o último ano, este paciente então deverá ser incluído na categoria de 1 a 3). Este campo não se aplica para pacientes com idade inferior a 7 anos. Em seguida, assinalar o maior grau de escolaridade concluído pelo paciente.
9. Anotar a atividade desempenhada pelo paciente no setor formal, informal ou autônomo. Em caso de afastamento temporário ou desemprego, considerar a última atividade exercida pelo paciente. Marcar, em seguida, a situação atual do paciente referente ao exercício de sua atividade.
10. Assinalar a renda mensal do núcleo familiar do paciente, considerando o somatório da renda de todos os membros da família, que residem no mesmo domicílio que o paciente.
11. Assinalar se o núcleo familiar do paciente recebe algum tipo de transferência governamental, incluindo aposentadoria, pensão, ou programa oficial de auxílio. Em resposta positiva, deve-se especificar o tipo de transferência. (Ex.: Bolsa-família; Bolsa-escola).
12. Assinalar se o paciente já vivenciou a fome, em algum momento de sua vida.
13. Anotar o tipo de logradouro (Ex.: Avenida, Rua, Travessa), nome completo e o número da residência do paciente, além de um ponto de referência.
14. Anotar o bairro, o município e a sigla do Estado onde o paciente reside.
15. Anotar o código de endereçamento postal do logradouro da residência do paciente.
16. Assinalar o tipo de zona da residência do paciente. Deve-se considerar zona urbana, a área com características estritamente urbana; rural, área com características estritamente rural e urbana/rural, a área com aglomeração populacional que se assemelha à área urbana.
17. Anotar o código de área e o número de telefone residencial, celular ou de um contato próximo do paciente.
18. Assinalar se a residência do paciente foi georreferenciada. Em resposta positiva, registrar os pontos de latitude e longitude.
19. Anotar há quanto tempo o paciente reside no domicílio atual.
20. Anotar o número de cômodos existente na residência do paciente, desconsiderando o número de banheiros.
21. Anotar o número total de moradores da residência.
22. Assinalar se na residência, há pelo menos um dormitório ocupado por mais de duas pessoas ao mesmo tempo.
23. Assinalar o bairro, o município, a sigla do Estado e o tempo de moradia na última e na penúltima residência do paciente, respectivamente.
24. Assinalar se o paciente tem expectativa de mudança próxima. Em resposta positiva, anotar o bairro, o município e a sigla do Estado de destino.
25. Assinalar a procedência do abastecimento de água na residência do paciente.
26. Assinalar o tipo de água utilizada para consumo pelo paciente.
27. Assinalar o tipo de rede de esgoto presente na residência do paciente.
28. Assinalar o destino do lixo utilizado na residência do paciente.
29. Assinalar se a residência do paciente é considerada subnormal ou não. O domicílio subnormal é aquela habitação carente dos serviços públicos e que não apresenta condições físicas para a moradia e, geralmente, está localizada em área irregular (propriedade particular, pública ou de preservação ambiental), a exemplo de invasões, favelas, assentamentos, palafitas, etc.
30. Assinalar se o paciente já conviveu, previamente, com algum caso de hanseníase. Em resposta positiva, marcar o número total de contatos e, em seguida, anotar o nome completo dos três contatos mais próximos e freqüentes relatados pelo paciente, selecionando o grau de parentesco, o tipo de convívio, o período de convivência e se a mesma ocorreu há mais de 10 anos.
31. Anotar a data em que o paciente foi diagnosticado com hanseníase. Em caso de recidiva, anotar a data do diagnóstico atual.
32. Anotar o nome completo da Unidade de Saúde em que foi realizado o diagnóstico.
33. Anotar o número de prontuário do paciente na Unidade de Saúde.
34. Anotar o número do SINAN do paciente.
35. Assinalar se o paciente é um caso de recidiva ou não.
36. Assinalar se o paciente foi fotografado ou não. Para resposta positiva, anotar o número de registro das imagens.
37. Assinalar o grau de incapacidade física resultante da avaliação por ocasião do diagnóstico, segundo as normas técnicas vigentes.
38. Assinalar o número de cicatrizes resultantes da vacina BCG. A cicatriz pode ser observada no braço direito do paciente. Em caso de dúvida quanto a sua presença, assinalar a categoria intitulada duvidosa. Na presença da cicatriz, medir com uma régua milimetrada transparente, o seu diâmetro transverso e longitudinal. Fazer a média dos dois resultados e anotá-la com a unidade mm.
39. Marcar no esquema do corpo humano, os tipos de lesões nas posições correspondentes às observadas no paciente no momento do diagnóstico, utilizando suas respectivas siglas.
40. Assinalar o número de lesões apresentadas pelo paciente no momento do diagnóstico.
41. Assinalar a classificação clínica do paciente, segundo Ridley-Jopling, no momento do diagnóstico.

INSTRUÇÕES PARA PREENCHIMENTO

42. Assinalar a classificação operacional do paciente atribuída, segundo normas técnicas vigentes, no momento do diagnóstico.
43. Assinalar a presença ou ausência de diabetes mellitus, hipertensão arterial sistêmica e neoplasias, concomitantes à hanseníase. Caso o paciente apresente outras doenças não mencionadas, deve-se anotá-las.
44. Assinalar a presença ou ausência de co-infecções por tuberculose, HIV e hepatite C. Caso o paciente apresente outras infecções não mencionadas, deve-se registrá-las.
45. Assinalar o resultado positivo ou negativo da baciloscopia do paciente, no momento do diagnóstico, ou se for o caso, a sua não realização.
    Para o resultado positivo de baciloscopia, registrar os índices morfológico(IM) e baciloscópico(IB), assim como, a data, o local onde a baciloscopia fora realizada e o nome do técnico responsável pela execução do teste (não rubricar).
46. Assinalar a forma clínica do paciente indicada pela histopatologia, na ocasião do diagnóstico, ou a sua não realização.
47. Anotar o resultado do ensaio imunoenzimático Anti-PGL1 do paciente, bem como os valores de densidade óptica, de cut-off, a data, o local onde o teste foi realizado, além do nome do técnico responsável ensaio (não rubricar).
48. Assinalar a presença ou ausência de mutações nos genes rpoB, folP e gyrA, ou a não realização do teste.
49. Anotar os resultados de genotipagem do Mycobaterium leprae ou assinalar a não realização do teste.
50. Assinalar o resultado positivo, negativo ou a não realização do diagnóstico molecular.
51. Assinalar o esquema terapêutico estabelecido para o paciente, por ocasião do diagnóstico. Em caso de tratamento alternativo, deve-se especificá-lo.
52. Assinalar a presença ou a ausência de reação hansênica antes de iniciar o tratamento. Em respostas positivas, deve-se marcar o tipo de reação apresentada pelo paciente, assim como o tratamento que lhe fora atribuído.
    *Assinalar a procedência das informações solicitadas ao paciente para responder as questões de número 53 a 58.
53. Anotar a data do primeiro diagnóstico de hanseníase do paciente.
54. Registrar o número de episódios de recidiva apresentados pelo paciente.
55. Anotar o nome completo da Unidade de Saúde (US) em que foi realizado o primeiro diagnóstico de hanseníase do paciente e, em seguida, assinalar se esta é a mesma US atual, outra ou de referência.
56. Marcar os tipos de lesões encontradas no paciente, utilizando suas respectivas siglas, no esquema do corpo humano, em posições correspondentes às relatadas no primeiro diagnóstico do paciente.
57. Assinalar o número de lesões apresentadas pelo paciente em seu primeiro diagnóstico.
58. Assinalar se as lesões apresentadas no primeiro diagnóstico desapareceram após o tratamento.
59. Assinalar a classificação clínica do paciente, segundo Ridley-Jopling, no primeiro diagnóstico.
60. Assinalar a classificação operacional do paciente, atribuída no primeiro diagnóstico do paciente, segundo normas técnicas vigentes.
61. Assinalar o esquema terapêutico estabelecido para o paciente, no primeiro diagnóstico. Em caso de tratamento alternativo, deve-se especificá-lo.
62. Anotar o grau de incapacidade física do paciente resultante da avaliação no primeiro diagnóstico.
63. Assinalar a presença ou a ausência de reação durante o tratamento inicial do paciente. Em resposta positiva, deve-se assinalar o tipo de reação, o tratamento atribuído e a qualidade da resposta a este tratamento.
64. Registrar o número de troncos nervosos afetados, na ocasião do primeiro diagnóstico. Em resposta positiva, assinalar quais troncos nervos foram afetados.
65. Assinalar o resultado positivo ou negativo da baciloscopia no primeiro diagnóstico do paciente, ou se for o caso, a sua não realização ou o desconhecimento do resultado.
66. Assinalar a forma clínica, segundo o resultado da histopatologia, no primeiro diagnóstico do paciente.
67. Assinalar a presença ou a ausência de reação no período pós-alta do primeiro diagnóstico do paciente. Em resposta positiva, deve-se assinalar o tipo de reação apresentada pelo paciente, o período de tempo entre a alta e o aparecimento de reação, o número de episódios reacionais, o tratamento atribuído, a continuidade deste tratamento e a qualidade da resposta do paciente.
68. Assinalar a presença ou a ausência de outras enfermidades após a alta do paciente. Em resposta positiva, deve-se especificá-las.
69. Anotar o nome completo das cinco pessoas que residem (ou residiram nos últimos 10 anos) com o paciente ou que mantiveram convivência próxima e freqüente. Em se tratando de paciente com recidiva, considerar os comunicantes desde o primeiro diagnóstico.
    Para cada comunicante registrado, deve-se selecionar o grau de parentesco, o período e o tipo de convivência estabelecida com o paciente. Para os comunicantes cuja convivência for extra-domiciliar, anotar o endereço, com ponto de referência e registrar os pontos de latitude e longitude do georreferenciamento da residência do paciente. Assinalar ainda, as informações requeridas referentes à cicatriz BCG, à forma clínica, à classificação operacional, à presença, ao número de lesões observadas e aos resultados da triagem sorológica e da confirmação molecular.
    Se o comunicante for considerado um caso novo, anotar o número de série do questionário.

APÊNDICE 2 – Ficha de identificação dos escolares.

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1 **Nome:**

2 **Nome da mãe:** _____

3 **Nome do pai:** _____

4 **Nascimento:** ____/____/____ 5 Idade:____ anos 6 Gênero: ☐ M ☐ F

7 **Naturalidade (Cidade/UF):** _____

8 **Cor da Pele/Etnia:** ☐ Negra ☐ Branca ☐ Parda ☐ Amarela ☐ Indígena

9 **Série atual:** _____

10 **Endereço:** _____

Ponto de referência: _____

11 **Bairro/Cidade/UF:** _____ 12 CEP: _____ - _____

13 **Fones:** (___) _____ Cel.: (___) _____ Contato: (___) _____

14 **Georreferencimento do domicílio**

☐ Não ☐ Sim Latitude: ____° ____' ____" Longitude: ____° ____' ____"

15 **Número de cicatrizes BCG:**

Número: ☐ 0 ☐ 1 ☒ 2 ☐ Duvidosa Diâmetro: 5,5 mm 3,5 mm

16 **Sorologia:**

ELISA (Anti-PGL1): _____ DO: _____ Cut-off: _____ ☐ Não realizada

17 **Diagnóstico molecular:** ☐ Positivo ☐ Negativo ☐ Não realizado

18 **Sensibilidade medicamentosa - Sequenciamento:**

rpoB mutação ☐ Não ☐ Sim ☐ Não realizado

folP mutação ☐ Não ☐ Sim ☐ Não realizado

gyrA mutação ☐ Não ☐ Sim ☐ Não realizado

19 **Genotipagem:**

_____ ☐ Não realizada

APÊNDICE 3 – Termo de consentimento livre e esclarecido dos escolares.

LABORATÓRIO DE DERMATO-IMUNOLOGIA

E 0901

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DO PARÁ - URE "MARCELLO CANDIA".

A equipe de pesquisadores coordenada pelo Professor Dr. Claudio Guedes Salgado está realizando o estudo **"Detecção e análise da variação genotípica do Mycobacterium leprae de casos-índice e de comunicantes, em regiões endêmicas do Estado do Pará"**, do qual gostaríamos que seu filho(a) ou menor sob sua responsabilidade participasse e, para isso, precisamos de sua autorização.

O estudo tem por objetivo identificar características relacionadas às pessoas, aos seus modos de vida, ao meio ambiente e à genética do micróbio que causa a hanseníase, para entender melhor sobre a transmissão da doença, melhorando sua prevenção e tratamento.

As crianças e os adolescentes que participarem deste estudo, serão submetidas à coleta de sangue e de secreção nasal, realizada por médicos e profissionais de saúde experientes, sempre com a finalidade de identificar o M. leprae. Suas casas serão visitadas, para que seja feito um mapeamento dos casos de hanseníase por bairros e ruas.

A pesquisa será realizada em ambiente seguro, com material limpo e descartável. A coleta de sangue poderá causar dor e pequena reação no local. E a coleta de secreção nasal será realizada de forma rápida e indolor.

A participação nesta pesquisa é voluntária e seu filho participará apenas se você autorizar. Sempre que desejar, será fornecido esclarecimentos sobre cada uma das etapas do estudo. A qualquer momento, o participante poderá recusar-se a continuar no estudo e retirar seu consentimento, sem que isso lhe traga qualquer penalidade ou prejuízo.

Está garantido o cumprimento do que fora informado acima, a gratuidade de todos os procedimentos e o sigilo da identidade dos participantes.

DECLARAÇÃO DE PAIS OU RESPONSÁVEIS

Tendo compreendido perfeitamente tudo o que me foi informado, AUTORIZO QUE MEU FILHO(A) _____ , PARTICIPE DESTE ESTUDO, SEM QUE PARA ISSO EU TENHA SIDO FORÇADO OU OBRIGADO.

_____

Assinatura ou impressão dactiloscópica do(a) pai/mãe ou responsável legal.

Doc.:_____

Local: _____ , Data:_____ /_____ /_____

Prof. Dr. Claudio Guedes Salgado
CRM-PA 5062
Laboratório de Dermato-Imunologia
Av. João Paulo II, 113. Marituba, Pará, Brasil. Fone: (91) 3256-9097


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