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TESE DE DOUTORADO

AVALIAÇÃO DOS TÍTULOS DOS ANTICORPOS ANTI-*LEPTOSPIRA* EM UMA COMUNIDADE ENDÊMICA DA CIDADE DE SALVADOR-BA: ASSOCIAÇÕES COM ASPECTOS EPIDEMIOLÓGICOS

JAQUELINE SILVA CRUZ

Salvador – Bahia

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Tese apresentada ao Curso de Pós- graduação em Biotecnologia em Saúde e Medicina Investigativa para obtenção do grau de Doutora.

Orientador: Prof. Dr. Mitermayer Galvão dos Reis

Coorientação: Dr. Elsio Augusto Wunder Júnior

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“Não há nada melhor do que despertar o prazer e o amor pelo estudo, caso contrário só se formam bons carregadores de livros”.

(**Michel Eyquem de Montaigne**)

CRUZ, Jaqueline Silva. **Avaliação dos títulos dos anticorpos anti-leptospira em uma comunidade endêmica da cidade de Salvador-BA**: associações com aspectos epidemiológicos**.** 2021. 78 f. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 2021.

RESUMO

INTRODUÇÃO: A leptospirose é uma zoonose de ocorrência mundial causada por bactérias do gênero *Leptospira.* A exposição ao ambiente contaminado com leptospiras é frequente em indivíduos de regiões endêmicas sendo agravado após fortes períodos sazonais de chuvas. A leptospirose apresenta manifestações clínicas que variam de uma infecção assintomática a doença grave. Atualmente, há poucas informações sobre a resposta imune protetora adquirida naturalmente contra infecção e reinfecção, o que dificulta o desenvolvimento de uma vacina eficaz e duradora contra a leptospirose. **OBJETIVO**: Avaliar a resposta imune humoral após infecção e reinfecção natural por *Leptospira* em indivíduos de uma região endêmica para leptospirose em Salvador, Bahia. **MATERIAIS E MÉTODOS**: Foi realizado um estudo de coorte com acompanhamento semestral na comunidade de Pau da Lima, Salvador-BA. Durante as visitas de acompanhamento, foram coletados dados epidemiológicos, sociodemográficos e coleta de sangue para realização do teste de microaglutinação (MAT) e avaliações imunológicas. A equação de estimativa generalizada foi utilizada para avaliar a associação entre precipitação e infecção por leptospiras. Um modelo matemático foi usado para avaliar a taxa de decaimento de títulos de anticorpos em indivíduos com infecção subclínicas ou assintomáticas. Além disso, realizamos o acompanhamento sorológico trimestral em uma subcoorte com 72 indivíduos para avaliara a cinética de anticorpos. **RESULTADOS**: Observamos que diferente do que ocorre com os casos hospitalizados, o risco de infecção por *Leptospira* teve uma associação inversa com a precipitação cumulativa. Na avaliação do decaimento de títulos do MAT, observamos aumento da taxa média de infecção com intervalo de seis meses. Sendo que houve um aumento ainda maior dessa taxa com intervalo de doze meses quando comparados à taxa convencional que não leva em consideração o decaimento de título. Na subcoorte, identificamos que 65% dos indivíduos apresentavam anticorpos anti-*Leptospira*. As reinfecções (n=25) foram mais frequentes quando se utilizou as avaliações trimestrais como referência. Além disso, os fatores de risco idade e atividades de risco como limpeza de esgoto estavam associadas a maior chance de adquirir infecções em ambas as análises com diferentes tempos de coletas. **CONCLUSÕES**: O presente estudo demonstrou que as infecções por *Leptospira* ocorrem durante todo ano devido a constante exposição desses indivíduos ao ambiente contaminado, que a resposta humoral mediante anticorpos aglutinantes é curta e protege parcialmente contra reinfecção.

Palavras-chave: Leptospirose. Imunidade humoral. Anticorpos.

CRUZ, Jaqueline Silva. **Evaluation of anti-leptospira antibody titers in an endemic community in the city of Salvador-BA:** associations with epidemiological aspects**.** 2021. 78 f. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 2021.

ABSTRACT

INTRODUCTION: Leptospirosis is a worldwide zoonosis caused by bacteria of the *Leptospira* genus. Exposure to an environment contaminated with leptospires is frequent for individuals from endemic regions, being aggravated after heavy seasonal periods of rain. Leptospirosis has clinical manifestations that range from asymptomatic infection to severe disease. Currently, there is little information on the naturally acquired protective immune response against infection and reinfection, which makes the development of an effective and long-lasting vaccine against leptospirosis difficult. **OBJECTIVE**: To evaluate the humoral immune response after infection and natural reinfection by *Leptospira* in individuals from an endemic region for leptospirosis in Salvador, Bahia. **MATERIALS AND METHODS**: A cohort study was carried out with semiannual follow-up in the community of Pau da Lima, Salvador-BA. During follow-up visits, epidemiological, sociodemographic and blood sampling data were collected for the microagglutination test (MAT) and immunological assessments. The generalized estimation equation was used to assess the association between precipitation and leptospiral infection. A mathematical model was used to assess the rate of decay of antibody titers in subjects with subclinical or asymptomatic infection. In addition, we performed quarterly serological follow-up in a subcohort of 72 subjects to assess antibody kinetics. **RESULTS**: We observed that, unlike in hospitalized cases, the risk of *Leptospira* infection had an inverse association with cumulative precipitation. In assessing the decay of MAT titers, we observed an increase in the mean infection rate with an interval of six months. Since there was an even greater increase in this rate with a twelve-month interval when compared to the conventional rate that does not take into account the decay of security. In the subcohort, we identified that 65% of the individuals had anti-*Leptospira* antibodies. Reinfections (n=25) were more frequent when quarterly assessments were used as a reference. In addition, age risk factors and risk activities such as sewage cleaning were associated with a greater chance of acquiring infections in both analyzes with different collection times. **CONCLUSIONS**: The present study demonstrated that Leptospira infections occur throughout the year due to the constant exposure of these individuals to the contaminated environment, that the humoral response through agglutinating antibodies is short and partially protects against reinfection.

Keywords: Leptospirosis. Humoral immunity. Antibodies.

LISTAS DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

BSA	Albumina sérica bovina
DNA	Ácido desoxirribonucleico
DPP	Plataforma de Duplo Percurso
ELISA	Ensaio Imunoenzimático
EMJH	Ellinghausen-McCullough-Johnson-Harris
FcpA	Proteína A de enrolamento flagelar
FcpB	Proteína B de enrolamento flagelar
GM-CSF	Fator estimulador de colônias de granulócitos-macrófagos
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IL	Interleucina
IL-1α	Interleucina 1 alfa
IL-1β	Interleucina 1 beta
IP-10	Interferon-gama induzido por proteína 10
Lig	Leptospiral immunoglobulin-like
LigA	*Immunoglobulin-like protein A*
LigB	*Immunoglobulin-like protein B*
LigC	*Immunoglobulin-like protein C*
LPS	Lipopolissacarídeo
LPHS	Síndrome da hemorragia pulmonar grave
MAT	Teste de aglutinação microscópica
MCP-1	Proteína-1 quimioatraente de monócitos
NRL	NOD-like receptors
OMS	Organização Mundial de Saúde
OMPs	Proteínas de mebrana externa
PCR	Reação em cadeia da polimerase
qPCR	Reação em cadeia da polimerase em tempo real
TNF-α	Fatores de necrose tumoral alfa
TRL	Toll-like receptors
VEGF	Fator de crescimento endotelial vascular

SUMÁRIO

1 **INTRODUÇÃO**

A leptospirose é uma zoonose de ampla distribuição mundial causada por espiroquetas patogênicas do gênero *Leptospira*, no qual encontram-se 17 espécies patogênicas e mais de 300 sorovares (FAINE et al., 1999; LEVETT et al., 2001; THIBEAUX et al., 2018; VINCENT et al., 2019). Animais sinantrópicos, domésticos e selvagens servem como reservatórios para abrigar a espiroqueta em seus túbulos renais e excretar em sua urina. A infecção em humanos ocorre após contato com água ou solo contaminados e varia de casos assintomáticos a uma doença febril aguda (BHARTI et al., 2003; LEVETT, 2001; MCBRIDE et al., 2005). A Organização Mundial de Saúde (OMS) estima que há mais de um milhão de casos humanos de leptospirose, com mais de 50.000 mortes a cada ano, a maioria dos quais ocorrem em países em desenvolvimento (COSTA et al., 2015) e países de clima tropical e subtropical (HAGAN et al., 2016).

A ocorrência de epidemias urbanas de leptospirose é impulsionada por fatores ambientais como chuvas intensas, umidade, temperatura, crescimento populacional, urbanização, falta de saneamento básico e alta infestação de roedores (COSTA et al., 2015; KO; GOARANT; PICARDEAU, 2009; LAU et al., 2010; SOO; KHAN; SIDDIQUI, 2020). Em regiões tropicais e áreas de alta prevalência, as fortes chuvas sazonais constituem condições favoráveis para que a *Leptospira* possa disseminar-se facilmente no ambiente através da água (FAINE et al., 1999; GANOZA et al., 2010; KO et al., 1999). O contato direto com água, solo ou vegetação contaminados com leptospiras tem sido amplamente documentados como fator de risco para a doença (SOO; KHAN; SIDDIQUI, 2020). Além disso, exposição a esgotos e lixo acumulado também tem sido associado a transmissão da doença no ambiente peridomiciliar (FELZEMBURGH et al., 2014; HAGAN et al., 2016; REIS et al., 2008).

A maioria das infecções causadas por *Leptospira* apresentam-se como formas assintomáticas, discretas ou casos mal diagnosticados, devido à ampla gama de sintomas que muitas vezes são confundidos com outras doenças como gripe, dengue e hepatite, levando a erros no diagnóstico o que não representa com precisão as taxas de incidência (CAUCHEMEZ et al., 2012; HARTSKEERL; COLLARES-PEREIRA; ELLIS, 2011). A sorologia pareada deve ser usada para identificar taxas de incidência precisas para essas infecções subnotificadas, e orientar as intervenções de saúde pública (ANDREWS; ITTYACHEN, 2018; WANGRANGSIMAKUL et al., 2018). Os métodos convencionais de interpretação de resultados de amostras sorológicas

pareadas não levam em consideração o decaimento dos títulos de anticorpos e como resultado, as taxas de infecção podem estar subestimadas, principalmente em estudos longitudinais onde há o potencial de reexposição destes indivíduos (CAUCHEMEZ et al., 2012; ZHAO et al., 2017).

A resposta imune humoral é o principal mecanismo de defesa contra a leptospirose e, é mediada pela produção de anticorpos circulantes direcionados contra o lipopolissacarídeo e limitada ao sorovar infectante (ADLER; DE LA PEÑA MOCTEZUMA, 2010). Indivíduos que vivem em regiões onde a leptospirose é endêmica estão frequentemente sendo expostos a *Leptospira*. Tem sido relatado a presença de anticorpos anti-*Leptospira* em pacientes que se recuperaram de doença grave e em indivíduos sem história prévia da doença, provavelmente resultante de infecção assintomática (TUERO; VINETZ; KLIMPEL, 2010). No entanto, há uma escassez de informações sobre os indivíduos que desenvolvem uma imunidade protetora adquirida naturalmente contra infecções ou doenças graves (TUERO; VINETZ; KLIMPEL, 2010).

É importante mencionar que até os dias atuais o papel da sazonalidade na transmissão da doença assintomática, o decaimento dos títulos de anticorpos e os mecanismos protetores envolvidos na infecção humana por *Leptospira* permanecem pouco conhecidos até os dias atuais. No presente estudo, avaliamos os títulos de anticorpos anti-*Leptospira* em moradores de uma comunidade urbana e associamos a aspectos epidemiológicos para obter informações relevantes que possam contribuir para o melhor entendimento da imunidade adquirida naturalmente contra leptospiras e para o desenvolvimento de vacinas mais eficazes.

2 **OBJETIVOS**

2.1 OBJETIVO GERAL

Verificar se os níveis de anticorpos aglutinantes anti-*Leptospira* se correlacionam com os aspectos socioepidemiológicos.

2.2 OBJETIVO ESPECÍFICOS

- Verificar se existe correlação entre a taxa de incidência de infecção por *Leptospira* e de casos hospitalizados com sazonalidade;
- Avaliar a taxa de decaimento de títulos de anticorpos anti-*Leptospira* em indivíduos de uma comunidade endêmica para leptospirose;
- Analisar se a cinética dos níveis de anticorpos aglutinantes após infecção por *Leptospira spp*. está associada a fatores de risco e de exposição.

3 **REVISÃO DE LITERATURA**

3.1 ETIOLOGIA E BIOLOGIA

As leptospiras são espiroquetas pertencentes ao filo Spirochaetales, família Leptospiraceae, com cerca de 0,1 mm de diâmetro por 6 a 20 mm de comprimento (JOHNSON & FAINE, 1984). Possuem extremidades em forma de gancho e dois flagelos periplasmáticos, responsáveis pela motilidade, compostos por proteínas FcpA, FcpB, FlaA e FlaB (PICARDEAU; BRENOT; SAINT GIRONS, 2001; WUNDER et al., 2016b, 2018).

As leptospiras são bactérias aeróbias obrigatórias com temperatura de crescimento entre 28 e 30° C e pH entre 7,2 a 7,6. (FAINE et al., 1999). Para seu crescimento e isolamento, é utilizado o meio de cultura Ellinghausen-McCullough-Johnson-Harris (EMJH) que é composto por Tween (ácido graxo, utilizados como fonte de carbono e metabolizados por β-oxidação), vitamina B1 e B12, sais de amônio e, albumina sérica bovina (BSA), sendo o último usado como agente detoxificante (ADLER; DE LA PEÑA MOCTEZUMA, 2010; EVANGELISTA; COBURN, 2010; FAINE et al., 1999). O gênero *Leptospira* é composto por espécies saprofíticas e patogênicas que apresentam crescimento lento e fastidioso (PICARDEAU, 2017). O tempo de geração das leptospiras varia de três horas para espécies saprófitas e de 8-18 horas para espécies patogênicas e o crescimento em meio de cultura pode variar de dois a 30 dias (FAINE et al., 1999).

3.2 TAXONOMIA

Para classificação do gênero *Leptospira*, são utilizados métodos sorológicos, com identificação de determinantes antigênicos e moleculares, baseados em análises do genoma (BRENNER et al., 1999).

A sorologia foi o método mais utilizado até o final da década de 80 (DIKKEN et al., 1979). Mediante métodos sorológicos, as leptospiras foram classificadas em mais de 260 sorovares que são agrupados em 24 sorogrupos. Esta classificação baseia-se na detecção de anticorpos aglutinantes antígeno-específicos e pelo teste de aglutinação microscópica (MAT). São agrupados no mesmo sorovar os isolados que apresentam reatividade cruzada com soro de animais identificados com sorovares determinados. Esta variação antigênica é atribuída principalmente à

estrutura do carboidrato do antígeno lipopolissacarídeo (LPS) (ADLER; DE LA PEÑA MOCTEZUMA, 2010; EVANGELISTA; COBURN, 2010).

Os métodos moleculares para identificação das leptospiras levam em consideração a análise filogenética dos genes que codificam a subunidade 16S rRNA. Recentemente, utilizando a análise filogenética, foi proposta uma nova classificação de 64 espécies de leptospiras divididas em quatro subclados: Espécies patogênicas (P1 e P2), e espécies saprófitas (S1 e S2), conforme descrito no Quadro 1 (VINCENT et al., 2019).

Quadro 1 - Descrição das espécies de *Leptospira spp*. de acordo com o subclado referente.

Subclados	**Espécies**
P1	*L*. interrogans, *L*. kirschneri, *L*. borgpetersenii, *L*. santarosai, *L*. noguchi, *L*. weilii, *L*. alexanderi, *L*. alstonii, *L*. kmetyi, *L*. adleri, *L*. ellisii, *L*. mayotetensis, *L*. barantonii, *L*. yasudae, *L*. stimsonii, *L*. gomenensis e *L*. tipperaryensis
P2	*L*. inadai, *L*. broomii, *L*. fainei, *L*. wolffii, *L*. licerasiae, *L*. perolatii, *L*. neocaledonica, *L*. hartskeerlii, *L*. haakeii, L. saintgironsiae, *L* venezuelensis, *L*. dzoumogneensis, *L*. fletcheri, *L*. fluminis, *L*. johnsonii, *L*. koniamboensis, *L*. langatensis, *L*. sarikeiensis, *L*. selangorensis, *L*. semungkisensis e *L*. andrefontaineae
S1	*L*. biflexa, *L*. wolbachii, *L*. meyeri, *L*. vanthielii, *L*. terpstrae, *L*. yanagawae, *L*. kemamanensis, *L*. brenneri, *L*. harrisiae, *L*. levettii, *L*. bandrabouensis, *L*. bourretii, *L*. bouyouniensis, *L*. congkakensis, *L*. ellinghoausenii, *L*. jelokensis, *L*. kanakyensis, *L*. montravelensis, *L*. mtsangambouensis, *L*. noumeaensis e *L*. perdikensis
S2	*L*. ilythenensis, *L* kobayashii, *L*. ognonensis, *L*. ryugenii e *L*. idonii

Fonte: Elaborado pela autora

3.3 **TRANSMISSÃO**

A transmissão da *Leptospira* pode ocorrer de duas formas: direta ou indireta. A transmissão direta, acontece quando fluídos corporais contendo *Leptospira spp.* passam diretamente de um

hospedeiro infectado para um hospedeiro susceptível (FAINE et al., 1999). Já a transmissão indireta ocorre quando um hospedeiro susceptível é infectado através de leptospiras disseminadas no ambiente através da urina de um animal infectado. O homem é considerado um hospedeiro acidental e terminal na cadeia de transmissão, sendo infectado pelo contato direto com sangue, órgãos ou urina, ou contato indireto com água ou solo contaminados pela urina ou outros fluídos dos animais portadores e reservatórios (KO, et al., 2009; MCBRIDE et al., 2005).

Um amplo espectro de animais sinantrópicos, domésticos e selvagens servem como reservatório de focos de infecção. Ao se infectarem, esses animais reservatórios não desenvolvem a doença e tornam-se portadores das leptospiras que colonizam os rins e são eliminadas vivas no meio ambiente através da urina, podendo sobreviver na água por vários meses (ANDRE-FONTAINE; AVIAT; THORIN, 2015). No meio urbano, os principais reservatórios são os roedores das espécies *Rattus norvegicus* (ratazana ou rato de esgoto), *Rattus rattus* (rato de telhado ou rato preto) e *Mus musculus* (camundongo ou catita); outros reservatórios são os suínos, bovinos, equinos, ovinos e cães (Figura 1). No Brasil, o *R. norvegicus* é o principal portador de cepas do sorogrupo *Icterohaemorrhagiae*, considerado um dos sorogrupos mais patogênicos para o homem (BRASIL, 2014).

Fatores como umidade, solo, água superficial e temperatura influenciam a sobrevivência das leptospiras no meio ambiente e, consequentemente, influenciam a transmissão da leptospirose (JOSHI; KIM; CHEONG, 2017; LAU et al., 2010; LEVETT, 2001). O solo, tem sido considerado como reservatório ambiental de transmissão da leptospirose em regiões endêmicas (CASANOVAS-MASSANA et al., 2018; SCHNEIDER et al., 2018; THIBEAUX et al., 2018).

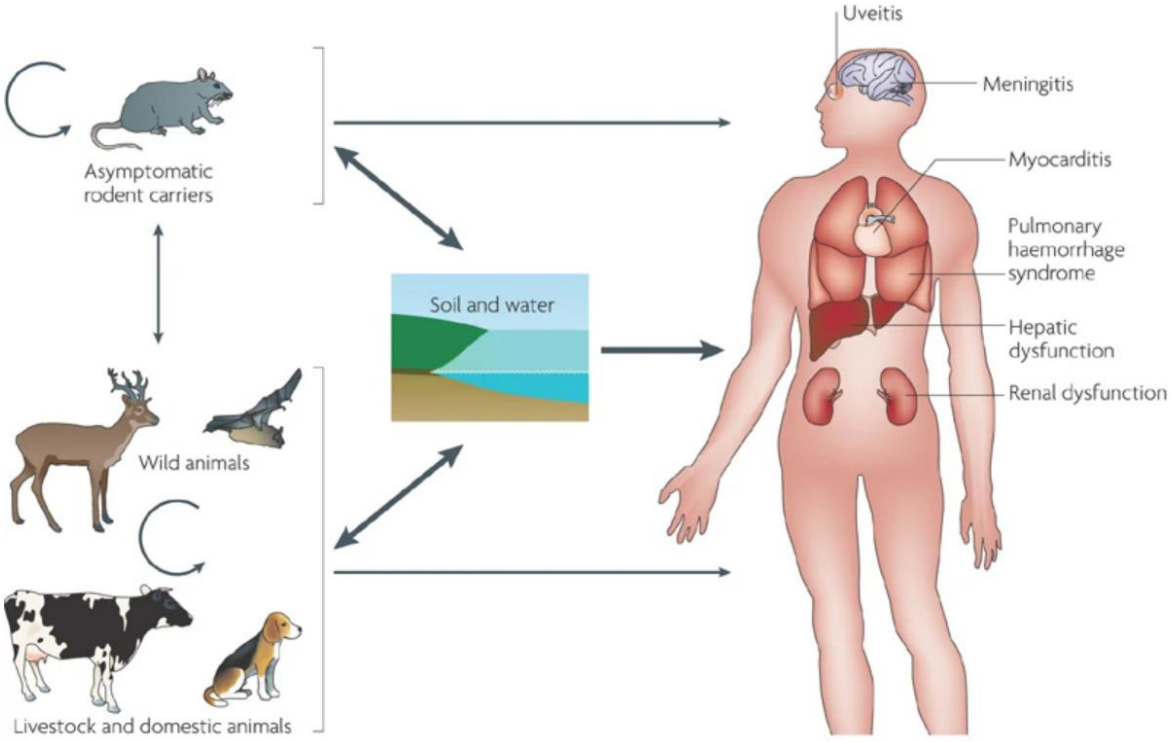

Figura 1 - **Ciclo de transmissão da leptospirose**. Várias espécies de animais domésticos e silvestres servem de reservatórios para a transmissão da *Leptospira* e que são excretadas através de sua urina. A doença é transmitida para humanos através do contato direto com animais reservatórios ou pela exposição à água de superfície ambiental ou solo contaminado com sua urina. Durante a fase leptospirêmica, a infecção causa uma doença febril aguda e que pode progredir para manifestações graves da doença como disfunção hepática e icterícia, insuficiência renal aguda, síndrome de hemorragia pulmonar, miocardite e meningoencefalite.
Fonte: (KO et al., 2009)

3.4 EPIDEMIOLOGIA

A leptospirose é a zoonose de maior distribuição geográfica mundial. A maior incidência ocorre em países de clima tropical, especialmente em países em desenvolvimento, na Ásia, Oceania, Índia, Caribe e América Latina. Nestas regiões, mudanças climáticas, aglomeração populacional, saneamento básico deficiente e grandes populações do hospedeiro são importantes fatores para o desenvolvimento da infecção (EVANGELISTA; COBURN, 2010; HAGAN et al., 2016; PAPPAS et al., 2008). Estima-se que anualmente ocorram mais de um milhão de casos de leptospirose no mundo com aproximadamente 59.000 mortes. A incidência nos trópicos é aproximadamente, 10 vezes maior do que em regiões temperadas (COSTA et al., 2015).

No Brasil, a leptospirose é considerada um importante problema de saúde pública, com uma média de 3.926 casos confirmados por ano, uma incidência de 1,02/100 habitantes e uma taxa média de letalidade de 8,9% (BRASIL, 2018). A doença ocorre em todo o país, principalmente nas

áreas urbanas vulneráveis sem infraestrutura sanitária adequada e com altas infestações de roedores, onde são frequentes as inundações durante as estações chuvosas. As inundações propiciam a disseminação e a sobrevivência de leptospiras no ambiente, favorecendo o contato do homem com água e solo contaminados e a ocorrência de surtos (KO et al., 1999).

3.5 ASPECTOS CLÍNICOS

Na leptospirose, observa-se uma variedade de manifestações clínicas. A gravidade da doença depende de fatores como virulência da cepa ou sorovar de *Leptospira* envolvido na infecção, bem como tamanho do inóculo, idade, estado de saúde e resposta imune do indivíduo infectado (EVANGELISTA; COBURN, 2010).

A grande maioria das infecções por *Leptospira* apresenta um quadro autolimitado e inespecífico. A fase aguda da doença pode durar de quatro a sete dias, e quando ocorre a leptospiremia, é caracterizada por febre, calafrios, cefaleia, mialgia e sufusão hemorrágica conjuntival, podendo ser confundido com outras doenças febris tais como dengue, Zika, Chikungunya, hepatite, influenza e hepatite (BHARTI et al., 2003; FAINE et al., 1999; PATTERSON; SAMMON; GARG, 2016). A fase imune inicia-se com o aparecimento de anticorpos aglutinantes da classe IgM e IgG de 5 a 14 dias após a exposição. As leptospiras são eliminadas da corrente sanguínea e dos órgãos à medida que aumentam os títulos dos anticorpos aglutinantes séricos (Figura 2) (BHARTI et al., 2003; FAINE et al., 1999; KO; GOARANT; PICARDEAU, 2009).

Entre 5-15% dos indivíduos infectados desenvolvem manifestações graves da doença, como a síndrome de Weil, caracterizada por icterícia, insuficiência hepática e renal, hemorragia e colapso cardiovascular, acarretando uma letalidade de 15% (BHARTI et al., 2003; EVANGELISTA; COBURN, 2010). Os casos mais graves da doença desenvolvem a síndrome de hemorragia pulmonar grave que se caracteriza pela lesão pulmonar aguda e sangramento pulmonar maciço. Em tais circunstâncias, a letalidade pode alcançar até 70% (GOUVEIA et al., 2008; HELMERHORST et al., 2012; KO; GOARANT; PICARDEAU, 2009). Nestes casos, pode ser necessário o tratamento intensivo, incluindo hemodiálise, transfusões sanguíneas e ventilação mecânica (GOARANT, 2016; KO; GOARANT; PICARDEAU, 2009).

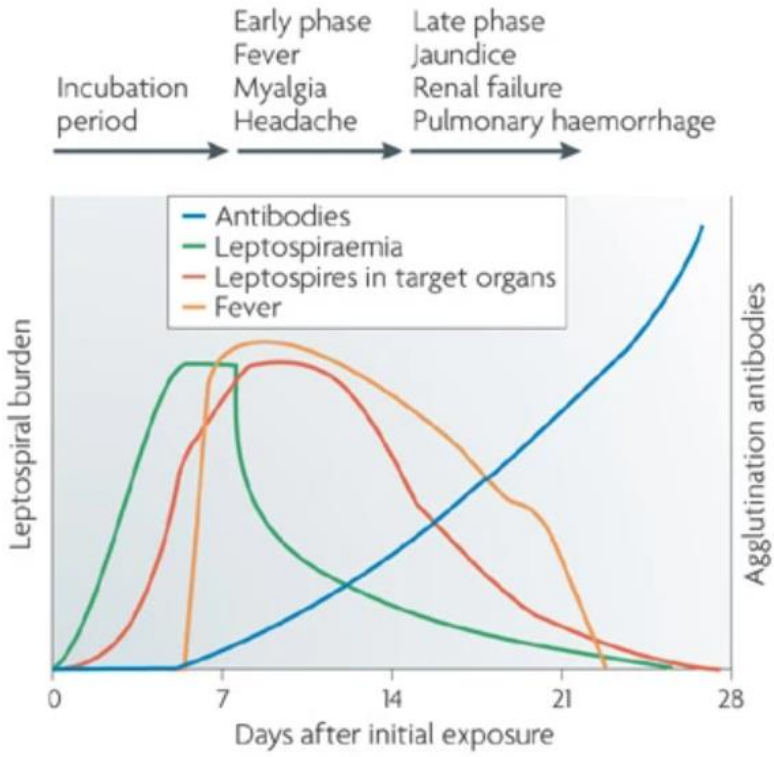

Figura 2 - Cinética da infecção. A infecção vai produzir leptospiremia nos primeiros dias após exposição e no terceiro dia de infecção, ocorre a migração das leptospiras para tecidos de múltiplos órgãos. Em humanos, na fase inicial da doença, desenvolve-se febre, mialgia e dor de cabeça. Os anticorpos aglutinantes aparecem de 5 a 14 dias após a exposição e à medida que aumentam, as leptospiras são eliminadas da corrente sanguínea e dos órgãos. Para a maioria dos indivíduos, a doença se apresenta de forma leve, porém uma pequena fração destes indivíduos, desenvolvem as manifestações graves de fase tardia, com icterícia, insuficiência renal e hemorragia pulmonar, de quatro a seis dias após início da doença.
Fonte: (KO, et al., 2009)

3.6 DIAGNÓSTICO

A leptospirose tem uma apresentação clínica semelhante a outras doenças febris agudas, e seu diagnóstico é dependente de exames laboratoriais para confirmação da infecção. Atualmente, os testes específicos mais utilizados para o diagnóstico da leptospirose incluem a cultura para o isolamento de leptospiras, sorologia e ensaios moleculares (BHARTI et al., 2003).

A cultura para leptospirose consiste em isolar leptospiras diretamente do sangue, tecidos e/ou fluídos de um indivíduo infectado. O momento ideal para atingir maior sensibilidade no procedimento de isolamento é obter amostras até 7-10 dias de sintomas. Porém, como a *Leptospira spp*. possui um crescimento fastidioso, é necessário que os tubos sejam examinados semanalmente

por 13 semanas. Por esta razão, são grandes as chances de contaminação por outros microrganismos e baixa sensibilidade (14,3-50%) (BROWN et al., 2003; TRUCCOLO et al., 2006). Além disso, a coleta da amostra para inoculação em meio EMJH deve ser feita antes do início do tratamento com antibióticos (BHARTI et al., 2003; LEVETT et al., 2001; MUSSO; LA SCOLA, 2013).

O MAT, é o método diagnóstico "padrão ouro" recomendado pela OMS, no qual se baseia na reatividade dos anticorpos dos soros dos pacientes contra antígenos de superfície da *Leptospira* (BHARTI et al., 2003), permitindo que seja identificado o sorogrupo infectante. Este teste não discrimina se os anticorpos produzidos são decorrentes de uma infecção ou de uma vacinação (ADLER; DE LA PEÑA MOCTEZUMA, 2010; PICARDEAU, 2013), um problema maior no caso de animais onde a vacinação é comum, já que vacinas para humanos não estão disponíveis (KO; GOARANT; PICARDEAU, 2009). Este teste é considerado como de difícil padronização e interpretação, exigindo experiência do profissional. Possui baixa sensibilidade, variando de 30 a 48.7% no início da infecção e preferencialmente requer amostras pareadas (CUMBERLAND; EVERARD; LEVETT, 1999; EUGENE et al., 2015; PICARDEAU et al., 2014). Por essa razão o seu uso é muito mais pertinente em estudos epidemiológicos do que o diagnóstico clínico.

O ELISA (do inglês Enzyme-Linked Immunosorbent Assay) que detecta IgM é um teste de fácil execução que detecta anticorpos mais precocemente que o MAT, a partir do sexto dia após o início dos sintomas (WHO, 2003). Este teste se baseia na detecção de anticorpos contra o extrato total de leptospiras, normalmente da cepa saprofítica *L. biflexa* sorovar Patoc, que compartilha vários antígenos de superfície com cepas patogênicas. Testes contendo o extrato celular de uma cepa epidêmica ou proteínas recombinantes também podem ser utilizados (LEVETT et al., 2001; PICARDEAU, 2013). Diversos kits comerciais encontram-se disponíveis no mercado e a sensibilidade e especificidade podem variar entre 31-76% e 66-98%, respectivamente (MUSSO; LA SCOLA, 2013).

Atualmente, existe necessidade de testes rápidos para o diagnóstico da leptospirose, permitindo o início da intervenção terapêutica imediatamente após o atendimento ao indivíduo infectado. O nosso grupo desenvolveu um teste rápido para leptospirose utilizando a metodologia *Dual Path Platform (DPP)* com a sensibilidade e especificidade variando entre 77-93% e 80-98% para pacientes com leptospirose grave, respectivamente (NABITY et al., 2012, 2018).

Mais recentemente, esforços foram realizados para o desenvolvimento de técnicas moleculares baseados no PCR convencional e em tempo real (qPCR) para amplificação do DNA

da bactéria (PEREZ; GOARANT, 2010; THAIPADUNPANIT et al., 2011). Muitos protocolos de PCR empregam diferentes combinações de oligonucleotídeos para detecção de DNA da *Leptospira spp.* em materiais clínicos (WAGGONER; PINSKY, 2016). Um resultado positivo na qPCR revela a presença do DNA do agente infeccioso na amostra, porém esta metodologia sozinha não permite que o sorovar infectante seja identificado. A sensibilidade do teste pode ser afetada devido à fraca ou curta leptospiremia na fase aguda, coleta tardia do material biológico ou administração de antibióticos (PICARDEAU, 2013).

3.7 TRATAMENTO

O tratamento da leptospirose envolve principalmente uma variedade de antibióticos administrados por via oral ou intravenosa (YAAKOB; FRANCIS RODRIGUES; VANITHA JOHN, [s.d.]). Antibióticos incluem tetraciclinas, penicilina e ceftriaxona, (BRETT-MAJOR; COLDREN, 2012) sendo a penicilina a mais utilizada (CHARAN et al., 2013). A administração precoce desses antibióticos reduz o risco da doença e a mortalidade nas formas graves da doença (TUBIANA et al., 2013). Nos casos graves, quando há complicações renais, respiratórias ou hemorrágicas, os pacientes devem ser encaminhados para hospitais que disponham de suportes como hemodiálise e ventilação mecânica (HAAKE, 2015; LEVETT, 2001).

3.8 PATOGENIA

A *Leptospira* penetra no hospedeiro através da pele lesionada, mucosas dos olhos, nariz ou garganta e estabelece uma infecção sistêmica por disseminação hematogênica, que envolve a passagem por muitas barreiras, incluindo a matriz extracelular, membranas basais e camadas celulares (KO; GOARANT; PICARDEAU, 2009; MURRAY, 2015). Esta penetração é favorecida pelo movimento translacional das bactérias (WUNDER et al., 2016a) e colonização, a qual se dá via interação das leptospiras com componentes da matriz extracelular e células do hospedeiro (EVANGELISTA et al., 2014). Mediante estudos experimentais com hamsters, têm-se demonstrado que a *Leptospira* pode atingir todos os órgãos, incluindo o cérebro, dentro de uma hora após a infecção (WUNDER et al., 2016a). A motilidade é essencial para a patogênese da doença, especialmente para atravessar as barreiras epidérmicas e de mucosa. Porém, considerando

que espécies saprófitas possuem flagelo e motilidade, outras propriedades do agente patogênico tem um papel essencial (WUNDER et al., 2016b, 2018).

As leptospiras penetram na corrente sanguínea e persistem até que o hospedeiro apresente uma resposta imune efetiva, que costuma ocorrer uma a duas semanas após a exposição. Entretanto, com o desenvolvimento da imunidade protetora, pode ocorrer a eliminação do agente ou o desenvolvimento do estado de portador crônico. Estudos mostram que o lúmen dos túbulos renais, onde a concentração de anticorpos é baixa, é o local de colonização ideal para as leptospiras, sendo provavelmente uma forma de escape do sistema imune (ATHANAZIO et al., 2008; FAINE et al., 1999).

Diversos fatores de virulência da *Leptospira* já foram identificados, entre eles destaca-se os LPS, que é considerado como fator geral de virulência de bactérias Gram-negativas, hemolisinas, proteínas da membrana externa (OMPs) e outras proteínas de superfícies, com potencial contribuição para a patogênese da infecção e doença (BHARTI et al., 2003; EVANGELISTA; COBURN, 2010). O LPS da *Leptospira* spp. exibe similaridade tanto estrutural quanto imunológica com LPS de bactérias Gram-negativas. Os anticorpos produzidos durante a infecção por leptospiras são aglutinantes e reativos, sobretudo, ao LPS bacteriano (EVANGELISTA; COBURN, 2010; FRAGA; BARBOSA; ISAAC, 2011; KO; GOARANT; PICARDEAU, 2009). A imunidade resultante de anticorpos reativos é limitada aos sorovares homólogos ou que estão intimamente relacionados e não se sabe se a resposta por anticorpos contra outros antígenos também confere proteção (FRAGA; BARBOSA; ISAAC, 2011; KO; GOARANT; PICARDEAU, 2009; LEVETT et al., 2001).

Além do LPS, proteínas estruturais e funcionais fazem parte da membrana externa das leptospiras, dentre elas destacam-se as *Ligs, LipL32 e Loa22* (Figura 3). As proteínas *Ligs* encontram-se expostas na superfície externa, incluindo *LigA, LigB e LigC*, sendo expressas apenas durante a infecção e estão envolvidas em processos de evasão imune e adesão das leptospiras (CHOY et al., 2007). Estas proteínas são capazes de induzir a produção de anticorpos específicos tanto em humanos como em animais infectados (MATSUNAGA et al., 2003; PALANIAPPAN et al., [s.d.]). Estudos apontam estas proteínas como potenciais candidatas à vacina para leptospirose, conferindo proteção em modelos animais entre 70-100% (FAISAL et al., 2009; YAN et al., 2009).

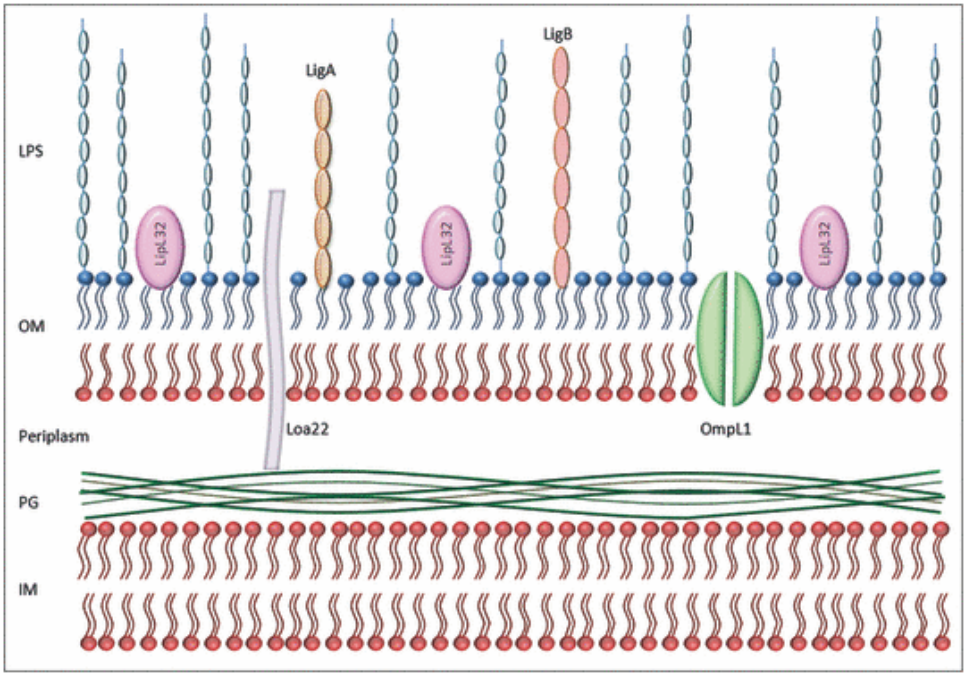

Figura 3 - Representação esquemática da membrana da espiroqueta. A membrana interna (IM) da *Leptospira* está ligada à parede celular de peptidoglicano (PG), que é sobreposta pela membrana externa (OM). Lipoproteínas expostas à superfície (LipL32, LigA, LigB e Loa22), a proteína de membrana externa transmembrana porina L1 (OmpL1) e o lipopolissacarídeo estão entre os principais componentes da membrana externa.
Fonte: (FRAGA, et. al.; 2011)

Outra proteína localizada na face externa da membrana (OM) da *Leptospira spp*. é a *Loa22*, que foi o primeiro fator de virulência identificado geneticamente e que preenche todos os postulados de Koch como fator de virulência. Os postulados de Koch são efetuados em quatro etapas: o microrganismo deve ser encontrado em todos os casos da doença; ser cultivado em cultura pura; provocar a doença quando inoculado num animal de experiência e poder ser isolado desses animais e cultivado em cultura pura. Cepas mutantes que exibem deleção do gene que codifica a *Loa22* apresentam virulência atenuada durante a infecção aguda em modelos experimentais (FALKOW, 2004; RISTOW et al., 2007; VIEIRA et al., 2012).

A *LipL32* é a proteína encontrada em maior abundância na superfície da *Leptospira* spp., sendo altamente imunogênica. Foi observado que mais de 95% dos infectados produzem anticorpos anti-*LipL32* (CULLEN; HAAKE; ADLER, 2004; GUERREIRO et al., 2001; HAAKE et al., 2000; MALMSTRÖM et al., 2009). Esta é uma proteína altamente conservada em espécies patogênicas e ausentes em cepas saprófitas, expressas durante a infecção aguda e que induz uma resposta imune humoral. Esta proteína apresenta grande potencial para imunodiagnóstico como antígeno vacinal

(ADLER; DE LA PEÑA MOCTEZUMA, 2010; BHARTI et al., 2003; HOKE et al., 2008; NALLY et al., 2007).

3.9 RESPOSTA IMUNE

O sistema imune inato compõe a primeira linha de defesa do hospedeiro, desempenhando um papel fundamental no reconhecimento e na eliminação das leptospiras. Durante as primeiras horas de infecção, a ativação da via alternativa do sistema complemento é um dos mecanismos efetores mais importantes (BARBOSA et al., 2009; MERI et al., 2005). A evasão ao sistema complemento por leptospiras patogênicas ocorre quando estas são capazes de se ligar ao Fator H, o qual, ligando-se a superfície das leptospiras, permanece ativo funcionalmente, atuando como cofator na clivagem do C3b pelo Fator I (FRAGA; BARBOSA; ISAAC, 2011; MERI et al., 2005). As leptospiras também são capazes de se ligar a C4bp, que é um inibidor da via clássica e das lectinas do complemento, no qual mantém sua atividade de cofator, atuando na clivagem de C4 mediada pelo Fator I. Isso indica que a aquisição deste fator regulador do complemento pode contribuir para a resistência ao soro pelas leptospiras (BARBOSA et al., 2009; DE SOUZA et al., 2016).

Receptores de reconhecimento de padrões, ou PRRs (do inglês – *pathogen recognition receptors*), são um dos principais mecanismos ativados que reconhecem antígenos de *Leptospira* spp., sendo os principais receptores pertencentes as famílias do tipo Toll e do tipo Nod. Estes parecem desempenhar papel relevante no reconhecimento das leptospiras pelo sistema imune inato do hospedeiro (EVANGELISTA; COBURN, 2010; FRAGA; BARBOSA; ISAAC, 2011). Já foi demonstrado que a presença dos receptores TRL2 e TRL4 é necessária para uma resposta imune inata efetiva contra a *Leptospira* spp. A ausência de TRL4 leva ao desenvolvimento da forma grave da doença com icterícia e hemorragia pulmonar, acúmulo de leptospiras nos rins e pulmões de camundongos infectados com *L. interrogans* sorovar Icterohaemorrhagiae (CHASSIN et al., 2009; WERTS et al., 2001).

O LPS das bactérias Gram-negativas ativa o TLR4, resultando em uma resposta pró-inflamatória mediada por citocinas e quimiocinas, já o LPS das leptospiras ativam os macrófagos humanos através do TLR2. Esta ativação diferenciada pode ser atribuída às diferenças na composição do lipídeo A como uma estratégia das espécies de leptospiras patogênicas para evitar

a ativação adequada de células do sistema imune, contribuindo assim para o estabelecimento da infecção em humanos (FRAGA; BARBOSA; ISAAC, 2011; GUERREIRO et al., 2001; NAHORI et al., 2005; QUE-GEWIRTH et al., 2004). Na ausência de estimulação dos TLR, receptores imune inatos da família Nod podem ser responsáveis por desencadear inflamação em resposta a leptospiras (CHASSIN et al., 2009).

Durante uma infecção, a resposta inflamatória é desencadeada após o contato com patógeno, que ativa o sistema imunológico inato. Estudos in vitro e em modelos animais demonstraram que leptospiras altamente virulentas podem induzir uma "tempestade" de citocinas nos estágios iniciais da enfermidade, com uma resposta tipo TH1, seguida de um estado de imunoparalisia que pode levar a sepse e falência de órgãos (CAGLIERO; VILLANUEVA; MATSUI, 2018; VERNEL-PAUILLAC; MERIEN, 2006). Em modelo animal, evidencia-se um padrão diferencial na expressão de citocinas, dependendo da resistência ou suscetibilidade. Em um estudo comparando camundongos e hamsters, foi observada uma rápida superexpressão da IL-10 anti-inflamatória em níveis mais altos em camundongos resistentes quando comparado ao observado em hamsters, independentemente da cepa de *Leptospira* (MATSUI et al., 2017). Vale ressaltar que em outro estudo para avaliar citocinas, foi observado a expressão significativamente maior de TNF-α, IL-1α e IL-10 nos hamsters infectados com doses letais de *L*. interrogans, quando comparados aos hamsters controle (VERNEL-PAUILLAC; GOARANT, 2010).

Em humanos, foi demonstrado que as concentrações das citocinas IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17 e TNF-α são significativamente maiores em indivíduos com quadros leves da doença, enquanto o baixo nível de TNF-α está relacionado com o desfecho fatal (REIS et al., 2013). Também já foi observado que os níveis de IL-6, IL-8, GM-CSF, IP-10, MCP-1 e VEGF diferiram significativamente entre os casos graves e o grupo controle, enquanto os níveis de GM-CSF diferiram significativamente entre os casos leves e o grupo controle (PAPA; KOTROTSIOU, 2015). Em outro estudo, as concentrações de IL-6, IL-8 e IL-10 estavam mais elevadas nos soros de indivíduos com comprometimento de órgãos em comparação com casos leves sem comprometimento de órgãos. Entretanto, somente o IL-8 foi significativamente maior em amostra de soro convalescente de indivíduos com comprometimento de órgãos (CHIRATHAWORN et al., 2016). Vale destacar que nos quadros assintomáticos de leptospirose, apresentou-se uma resposta anti-inflamatória com células TCD4+ mais produtoras de IL-10 em comparação com indivíduos com leptospirose grave ou leve (VOLZ et al., 2015).

Na leptospirose, a imunidade adquirida é predominantemente humoral e dependente da produção de anticorpos e da ativação da via clássica do sistema complemento sendo que a maioria dos anticorpos, são produzidos contra o LPS. Esta ativação promove a fagocitose da bactéria por neutrófilos e macrófagos. A fagocitose só ocorre quando a *Leptospira* spp. encontra-se opsonizada por anticorpos IgG específicos, sugerindo que o envelope das leptospiras possui um componente antifagocítico (FRAGA; BARBOSA; ISAAC, 2011; KO; GOARANT; PICARDEAU, 2009). Imunoglobulinas do tipo IgM são produzidas no início da infecção. Após alguns dias, há produção de imunoglobulinas do tipo IgG, que provocam a lise das leptospiras circulantes. Sugere-se que complexos imunes circulantes podem estar correlacionados com a intensidade dos sintomas, principalmente com o aumento da gravidade da doença de Weil, que pode ser resultante de uma resposta imune humoral intensa, já que falência renal, trombocitopenia e alterações pulmonares aparecem na fase imune da doença (GONÇALVES et al., 2014).

Em um estudo experimental realizado com cobaias, foi observado que o LPS desempenha um papel eficaz na prevenção da doença, com aumento significativo da taxa de sobrevivência de hamsters pré-tratados. Além disso, a carga de *Leptospira* e o grau de lesão patológica do rim, fígado e pulmão diminuíram significativamente quando comparados ao grupo controle. O pré-tratamento com LPS também aumentou a fagocitose e a capacidade bactericida dos macrófagos para *Leptospira* (CHEN et al., 2020). Nos estudos iniciais, foi demonstrado que o soro de pacientes exercia ação protetora em cobaias infectados, que o LPS é o principal alvo da produção de anticorpo na resposta protetora contra a *Leptospira* spp. (ADLER; FAINE, 1978) e que resposta humoral exerce uma função importante para o controle da infecção por leptospiras e medeia o mecanismo de resistência natural (FAINE et al., 1999). Esta resposta humoral é demonstrada pela presença de anticorpos das classes IgM e IgG no soro de indivíduos que convalesceram de formas graves da doença em até seis anos após a infecção inicial (EVANGELISTA; COBURN, 2010). Outro estudo sugere que a presença de perfil de anticorpos anti-*Leptospira* pré-existentes protegem contra o desenvolvimento da forma grave da leptospirose (LESSA-AQUINO et al., 2017).

O papel da imunidade celular na leptospirose ainda permanece pouco compreendido. Embora as leptospiras não sejam consideradas patógenos intracelulares, há relatos que a *L. Interrogans* é capaz de escapar do fagolisossoma para o citosol de macrófagos humanos (TANG, 2015). Os linfócitos T desempenham um papel importante na produção de IFN-ɣ, citocina que auxilia na ativação dos macrófagos e recrutamento de outros leucócitos para o local da infecção

(CHASSIN, 2009). Um estudo realizado para avaliar a resposta celular em sangue total ou células mononucleares do sangue periférico (PBMC) humano mostrou que a estimulação com *L. interrogans* morta leva a produção de IFN-ɣ, IL-12p40 e TNF-α por linfócitos T e células NK (DE FOST et al., 2003; TUERO; VINETZ; KLIMPEL, 2010), e que dentre os linfócitos T, há uma expansão predominante da população de linfócitos TCRɣδ, ao invés da população com TCRαβ. Os linfócitos T CD8 também podem ser ativados, mostrando especificidade para antígenos de superfície de *Leptospira*, como a proteína LigA (GUO; WANG; SUN, 2010).

No modelo murino, foi demonstrado que os leucócitos e linfócitos B e T são responsáveis pela inflamação independente do MyD88. Também foi sugerido que as células B são as principais responsáveis pela produção de INF-α dependente de TRL2 e TRL4 no fígado e que demonstra papel protetor das células B, dependente parcialmente da produção precoce de IgM dependente de TRL4, direcionada especificamente contra *Leptospira* spp. (CHASSIN et al., 2009).

Atualmente, um dos maiores desafios é o desenvolvimento de uma vacina universal para a leptospirose. As leptospiras desenvolveram mecanismos para escapar da função protetora do sistema do complemento, multiplicar-se no sangue, aderir às células do hospedeiro e penetrar nos tecidos em um ritmo mais rápido. A capacidade de colonizar rapidamente vários órgãos representa uma grande ameaça para o hospedeiro sendo a principal razão para a necessidade de desenvolver uma vacina efetiva contra a leptospirose (ADLER, 2015).

Até o presente momento, as vacinas comercialmente disponíveis contra leptospirose humana foram utilizadas para imunização em indivíduos com alto risco ocupacional, viajantes para regiões endêmicas da doença e/ou em resposta a enchentes e epidemias no Japão, Cuba, França e China. Apesar da utilidade, estas vacinas geralmente fornecem proteção homóloga a curto prazo contra os sorovares incluídos na preparação da vacina (ADLER, 2015; FRAGA; BARBOSA; ISAAC, 2011).

3.10 REINFECÇÃO

Até o momento, pouco se sabe sobre o papel da imunidade adquirida naturalmente à reinfecção (FELZEMBURGH et al., 2014; GRILLOVÁ et al., 2020; VIJAYACHARI et al., 2004). Estudos são necessários para um melhor entendimento de como, em alguns indivíduos, essas infecções repetidas ocorrem. Em um estudo realizado para identificar prospectivamente a infecção

por *Leptospira* entre crianças em idade escolar nas Ilhas Andaman em 2004, relatou-se uma taxa de infecção de 33,5% entre os soronegativos e a taxa de reinfeção encontrada foi de 16,7% entre os soropositivos durante o surto da doença ocorrido um mês após início do estudo (VIJAYACHARI et al., 2004).

Outro estudo relatou que anticorpos de uma primeira infecção pode não proteger contra uma reinfecção e que em um caso, a reinfecção ocorreu em menos de um ano, mostrando que anticorpos preexistentes de uma primeira infecção não conferiram proteção cruzada entre uma espécie de *Leptospira* não relacionada (*L*. interrogans vs. *L*. weili) (GRILLOVÁ et al., 2020).

No Brasil, um inquérito sorológico com 2.003 moradores de uma área endêmica para leptospirose na cidade de Salvador, Brasil, encontrou uma taxa de infecção secundária 2,3 vezes maior do que a taxa de infecção primária (71,7 e 31,1 infecções por 1.000 pessoas-ano) e que a exposição repetida à bactéria patogênica é um evento frequente entre os moradores desta comunidade (FELZEMBURGH et al., 2014).

4 **RESULTADOS**

Os resultados serão apresentados na forma de dois capítulos conforme apresentados a seguir:

4.1 CAPÍTULO 1

O artigo 1 “Influence of Rainfall on *Leptospira* Infection and Disease in a Tropical Urban Setting, Brazil” foi publicado na Emerging Infectious Diseases em fevereiro de 2020. Neste estudo foi avaliada a influência dos fatores meteorológicos nas taxas de infecções por leptospirose.

Influence of Rainfall on *Leptospira* Infection and Disease in a Tropical Urban Setting, Brazil

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The incidence of hospitalized leptospirosis patients was positively associated with increased precipitation in Salvador, Brazil. However, *Leptospira* infection risk among a cohort of city residents was inversely associated with rainfall. These findings indicate that, although heavy rainfall may increase severe illness, *Leptospira* exposures can occur year-round.

Leptospirosis, a leading zoonotic cause of illness and death (*1*), has emerged as a major health problem due to the global expansion of urban slum communities (*2*–*4*). The disease is associated with severe manifestations such as Weil's disease and pulmonary hemorrhage syndrome (*5*), for which case-fatality rates are 10%–50% or even higher (*6*). Transmission to slum residents occurs in the peridomiciliary environment, in which exposures to sewers, floodwater, and contaminated soil are risk factors (*3*,*7*,*8*). Extreme weather events may precipitate outbreaks (*3*–*6*), as recently experienced during the aftermath of Hurricane Maria in Puerto Rico (*9*). Similarly, seasonal periods of heavy rainfall and flooding are a contributing factor to the risk for urban leptospirosis (*4*,*10*).

In urban slum settings, contact with rats and *Leptospira*-contaminated water and soil occur year-round (*3*). Prior studies have shown, consistently, positive associations between heavy rainfall and hospitalized leptospirosis case-patients (*4*,*10*). However, this relationship may be affected by differences in case definitions used by diverse surveillance systems. In the few prospective cohort studies available, estimates of severe disease accounted for only a small proportion of the total disease burden (*6*). Thus, little is known about the role of rainfall in overall infection rates. To characterize the seasonal pattern of leptospirosis and *Leptospira* infection in a tropical urban setting and evaluate the influence of meteorological factors on seasonal risk, we conducted a prospective investigation of *Leptospira* infection rates among slum residents while actively surveying for hospitalized leptospirosis case-patients within Salvador, Brazil, during seasonal periods of high and low rainfall.

The Study

During February 2013–April 2015, we identified patients >5 years old with suspected leptospirosis at the state infectious disease hospital in Salvador, Brazil (*4*,*5*), and those reported in the public health surveillance database by other hospitals in Salvador. We estimated the probable date of infection as 15 days before the hospital admission date. We evaluated suspected leptospirosis cases according to the WHO case definition standard (*4*,*6*,*11*) using the microscopic agglutination test (MAT), lipL32 real-time PCR assay (*11*), IgM-ELISA (*6*), or a combination. We defined laboratory-confirmed cases of leptospirosis as those with >4-fold rise in MAT titers in paired serum samples, MAT titers >1:800 in a single sample, or positive PCR (Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/26/2/19-0102-App1.pdf).

A linear regression model identified that cumulative monthly rainfall (Figure 1, panel A) was significantly associated with the monthly number

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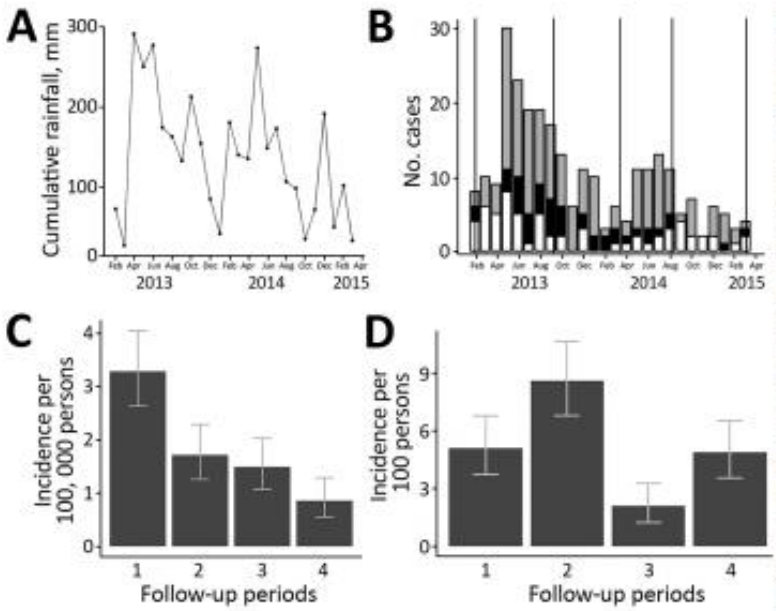

Figure 1. Temporal distributions of rainfall, cases of leptospirosis requiring hospitalization, and *Leptospira* infections in Salvador, Brazil, February 2013–March 2015. A) Cumulative monthly rainfall. B) Monthly citywide cases of leptospirosis requiring hospitalization, which were reported to the national surveillance system and stratified according to confirmed (black bar), probable (gray bars), and unconfirmed (white bars) case status. Vertical lines represent the dates the 5 serosurveys were performed during the 2-year study. C) Cumulative incidence of citywide cases of leptospirosis requiring hospitalization during 4 biannual follow-up periods for a community-based cohort. D) Cumulative incidence of *Leptospira* infection among a cohort of 861 residents of an urban slum community within Salvador during 4 biannual follow-up periods. Error bars in panels C and D indicate 95% CIs.

of hospitalized cases ($r^2 = 0.22$, $p<0.007$) (Figure 2). The highest hospitalized disease incidence occurred during the first period (February–September 2013; 3.29 cases/100,000 population; 95% CI 2.67–4.01 cases/100,000 population) and decreased across the next periods (Table 1; Figure 1, panels B, C).

Concurrently, we conducted a prospective cohort study assessing serologic evidence of *Leptospira* infection among urban slum residents of Pau da Lima, northwestern Salvador. We enrolled 2,421 of 3,716 eligible residents, ≥5 years of age and with written informed consent, of whom 821 participated in all serologic surveys performed twice annually during August–September (dry season) and February–March (rainy season) during 2013–2015 (Figure 1, panel A). Using panels with the 2 most common *Leptospira* species in Salvador (*4*), *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni (strain Fiocruz L130) and *L. kirschneri* serogroup Cynopteri serovar Cynopteri (strain 3522C), we defined serologic evidence of *Leptospira* infection by a MAT titer increase from negative to ≥1:50 (seroconversion) or ≥4-fold increase between sequential, paired samples. During the study period, 29% of the infected participants reported fever.

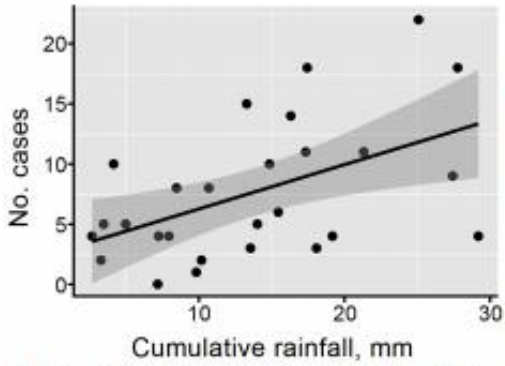

Figure 2. Correlation between cumulative monthly rainfall and monthly citywide cases of leptospirosis requiring hospitalization.

To assess the association between rainfall and laboratory-confirmed *Leptospira* infection, we calculated the cumulative amount of rainfall that each study participant experienced between sequential samples. We used a generalized estimating equation and incorporated explanatory variables for gender, age, time period, and cumulative rainfall that each participant experienced. In contrast to the hospitalized cases, we found *Leptospira* infection risk in the urban area had an inverse association with cumulative rainfall (0.986 cm, 95% CI 0.977–0.995 per cm) (Table 2; Figure 1, panel D). We additionally assessed various rainfall metrics, as well as the number of severe rainfall events each participant experienced above the mean rainfall, and the resulting patterns remained

Table 1. Cumulative rainfall, citywide incidence of leptospirosis requiring hospitalization, and incidence of *Leptospira* infection among a community-based cohort in Salvador, Brazil, 2013–2015*

Follow-up period (dates)*	Cumulative rainfall, cm (+ SD)†	Hospitalizations/100,000 population‡		*Leptospira* infection in period§	
		No. cases	Incidence (95% CI)	No. infected	Incidence (95% CI)
1 (2013 Feb 2–Sep 10)	126 (± 13)	88	3.29 (2.67–4.01)	44	5.11 (3.74–6.80)
2 (2013 Sep 10–2014 Mar 14)	81 (± 21)	46	1.72 (1.26–2.29)	74	8.60 (6.81–10.67)
3 (2014 Mar 14–2014 Aug 8)	93 (± 16)	40	1.50 (1.07–2.04)	18	2.09 (1.24–3.28)
4 (2014 Aug 8–2015 Mar 3)	57 (+ 11)	23	0.86 (0.54–1.29)	42	4.88 (3.54–6.54)

*We conducted 5 semiannual follow-up surveys for a community-based cohort of 861 residents of a community within Salvador, Brazil. A period was defined as the interval between 2 consecutive surveys.
†The source of rainfall data is 4 weather stations maintained by the Brazilian Institute for the Environment and Water Resources (Instituto do Meio Ambiente e Recursos Hídricos), located 1.6 km from the study site.
‡Cases of hospitalized leptospirosis per 100,000 population in the city of Salvador, Brazil (pop. 2,675,656 in 2010), during the follow-up period.
§We performed microscopic agglutination test to evaluate serologic evidence of *Leptospira* infections between 2 consecutive surveys. Cumulative incidence was calculated as the number of infections per 861 cohort subjects multiplied by 100.

consistent. Increasing age and male sex were associated with higher infection risk.

Conclusions

Leptospirosis is traditionally associated with heavy rainfall and flooding events in Brazil (*5*,*9*) and worldwide (*7*,*10*). Our findings support the association between extreme weather events and clinical leptospirosis. During the study period, the risk of acquiring leptospirosis that required hospitalization was significantly higher in periods with elevated rainfall. However, this finding is in contrast to *Leptospira* infection in nonhospitalized persons.

Our findings indicate that *Leptospira* infections occur year-round in this urban tropical setting and the cumulative incidence of *Leptospira* infection is high (2%–9% per period). This finding differs from patterns that we and others have identified for leptospirosis requiring hospitalization (*2*,*4*,*9*,*12*). Although this study does not specifically assess subclinical symptomatic infection, it provides further evidence that the impact of leptospirosis is underestimated, and physicians should be aware that leptospirosis infection may manifest clinically year-round.

The patterns of *Leptospira* exposure incidence and infection severe enough to require hospitalization, when taken together, suggest that rainfall may promote exposures of greater inocula, which in turn may increase the risk of developing severe clinical outcomes, such as severe pulmonary hemorrhage syndrome and Weil's disease. For example, heavy rainfall may diffuse *Leptospira* from the soil, resulting in higher concentrations of bacteria in the media to which humans are exposed (sewer water) and so to a higher inoculum dose, thus increasing hospitalized disease incidence and perhaps decreasing the environmental exposure risk in and around households (mud and exposed soil) and decreasing infection risk. However, additional studies are needed to assess the specific contribution of inoculum dose to disease severity.

The 2-year study period was atypical because rainfall was lower than expected during the rainy seasons (Figure 1, panel A; Appendix Figure 1). Of note, we observed a significant inverse association between cumulative rainfall and the risk for infection during biannual sampling periods. Thus, these trends may not apply to periods with higher amounts of rainfall or extreme climatic events, such as El Niño. This study was also limited because we used seroconversion to identify infection and therefore could not determine the precise timing of exposure events; furthermore, we conducted serologic surveys only in a single urban slum community. However, most hospitalized cases occur in similar communities (*4*), and therefore Pau da Lima is likely to be representative. Last, although the surveillance hospitals were able to capture a variety of febrile illnesses, they did not capture mild febrile illness, which may account for a missing proportion of leptospirosis cases.

Our findings demonstrate that, despite the association of leptospirosis hospitalization with rainfall, *Leptospira* exposure continues year-round. Although we did not evaluate mild subclinical or clinical infections, it is possible that participants experience symptomatic illness that may be unrecognized or misdiagnosed as dengue or other febrile disease (*12*,*13*). Clinicians should be aware that leptospirosis may

Table 2. Association of cumulative rainfall and semiannual follow-up period with risk for *Leptospira* infection, Salvador, Brazil, 2013–2015*

Variable	Odds ratio (95% CI)
Per year of age	1.02 (1.02–1.03)
Male sex	1.98 (1.48–2.64)
Cumulative rainfall, cm†	0.986 (0.977–0.995)
Period	
1	Referent
2	1.15 (0.63–2.10)
3	0.30 (0.15–0.59)
4	0.44 (0.20–0.97)

*We used Generalized Estimating Equation to evaluate the association of rainfall, follow-up period, and patient age and sex on *Leptospira* infection, as ascertained by serologic evidence, assuming a dependence on the individual level across the 4 repeated measures.
†Cumulative amount of rainfall experienced by participant between sequential samples.

manifest clinically outside of normal seasonal periods of heavy rainfall. In addition, the differences observed during the time periods independent from rainfall indicate that other unexplained factors may influence the temporal risk for *Leptospira* infection. Identifying these factors will help enhance intervention strategies in urban slum environments.

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4.2 CAPÍTULO 2

O capítulo 2 é composto por dois artigos:

Artigo 2: **“Effects of accounting for interval-censored antibody titer decay on seroincidence in a longitudinal cohort study of leptospirosis”** foi publicado na American Journal of Epidemiology em 04 de dezembro de 2020. Nesse artigo avaliamos a taxa de decaimento de títulos de anticorpos analisados em intervalos de coletas de seis e doze meses.

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Original Contribution

Effects of Accounting for Interval-Censored Antibody Titer Decay on Seroincidence in a Longitudinal Cohort Study of Leptospirosis

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Accurate measurements of seroincidence are critical for infections undercounted by reported cases, such as influenza, arboviral diseases, and leptospirosis. However, conventional methods of interpreting paired serological samples do not account for antibody titer decay, resulting in underestimated seroincidence rates. To improve interpretation of paired sera, we modeled exponential decay of interval-censored microscopic agglutination test titers using a historical data set of leptospirosis cases traced to a point source exposure in Italy in 1984. We then applied that decay rate to a longitudinal cohort study conducted in a high-transmission setting in Salvador, Brazil (2013–2015). We estimated a decay constant of 0.926 (95% confidence interval: 0.918, 0.934) titer dilutions per month. Accounting for decay in the cohort increased the mean infection rate to 1.21 times the conventionally defined rate over 6-month intervals (range, 1.10–1.36) and 1.82 times that rate over 12-month intervals (range, 1.65–2.07). Improved estimates of infection in longitudinal data have broad epidemiologic implications, including comparing studies with different sampling intervals, improving sample size estimation, and determining risk factors for infection and the role of acquired immunity. Our method of estimating and accounting for titer decay is generalizable to other infections defined using interval-censored serological assays.

4-fold rise; antibody; interval-censored assay; leptospirosis; paired serology; seroconversion; serological assay; titer decay

Abbreviation: MAT, microscopic agglutination test.

Infections with a high proportion of asymptomatic, mild, or misdiagnosed cases are not accurately represented by reported cases and require serological investigation (1). For example, patients with fevers of acute onset and/or unknown origin are often preliminarily diagnosed with well-recognized, highly endemic pathogens based on clinical presentation. However, when samples are subjected to serological analysis, lesser-known or emerging infections can account for many of these illnesses (2–6). Incidence rates are a crucial epidemiologic tool for investigation of an infection's burden, transmission dynamics, and trends, all of which guide public health interventions. It is therefore critical that paired serology be used to produce accurate incidence rates for these underreported infections, such as hantavirus, rickettsial disease, leptospirosis, and influenza, dengue, and Zika viruses (1–7).

Paired serology defines infections based on 2 samples from an individual (1). It is commonly used in longitudinal cohort studies. To conduct paired serology, 2 serum samples are obtained from an individual at different times. These samples are then subjected to a serological test, and the test values are compared to determine whether there is evidence that an infection has occurred. By long-standing convention, recent infections are defined according to 2 definitions: a seroconversion or a 4-fold rise in antibody titer (1). A seroconversion is defined when an individual with an initial antibody response below the test threshold has a positive result in the second. A 4-fold rise is defined when

an individual has an initial response above the detection threshold and a second result at least 4 times the first (for example, a titer of 50 followed by one of 200). This 4-fold rise is equivalent to a 2-dilution change in a serial dilution test. The 4-fold rise criterion stems from the variability in the performance and interpretation of serological assays, which implies that a 2-fold rise (a single dilution change) could represent measurement error, not infection (1). In longitudinal studies, these definitions are applied irrespective of the interval between samples.

Antibody decay is a known phenomenon (8–10) that would be expected to occur in the interval between samples but is not accounted for by conventional interpretations of paired sera; as a result, they might underestimate infection rates when applied to studies with long intersample intervals. Failing to account for the decay in an individual's antibody levels after the initial sample is taken means that the second sample is compared with an artificially high baseline value when applying the seroconversion or 4-fold rise criteria. This decreases the likelihood that an infection will be declared. It has been demonstrated that the conventional interpretations underestimate infections at the population level (1, 11), and some authors have suggested using a 2-fold rise in titer to define infections when estimating population-level attack rates (1, 11). However, this does not address individual-level measurement error and could therefore overestimate infection rates.

Interpretation of paired serology can be further complicated by 2 features shared by a number of pathogens. The first is the use of interval-censored serological assays, such as the hemagglutination-inhibition assay used for influenza diagnostics (1) or the dengue plaque reduction neutralization test (12). These tests do not produce a continuous numeric result but instead indicate an interval into which a sample's titer falls. The second is the potential for reexposure in longitudinal cohorts. Reexposure can boost titers, masking titer decay. One method of handling this is to model longitudinal data allowing for both infection and titer decay simultaneously (8, 9, 11, 13, 14), but the 2 processes can be statistically unidentifiable, preventing accurate estimation of either (15).

One pathogen for which seroincidence studies have been crucial is leptospirosis, a bacterial zoonosis. Pathogenic spirochetes of the genus *Leptospira* cause approximately 1 million severe cases and almost 60,000 deaths per year (16). Humans become infected through direct contact with an infected mammalian host or contact with soil or water contaminated by infected animal urine (17). Severe manifestations, including pulmonary hemorrhage and Weil's disease, represent only a small fraction of infections (17). The majority are asymptomatic or produce mild disease with nonspecific symptoms such as fever and myalgia (17) and are frequently misdiagnosed as better-known infections like malaria or dengue fever (3–6). The burden of leptospirosis is thus severely underestimated by reported cases, and seroincidence studies have been critical for understanding leptospiral dynamics and risk factors for infection (18, 19). As with other diseases, serological studies of leptospirosis are complicated by interval-censored titers and the potential for reexposure.

To characterize the effect of allowing for titer decay between serological samples, we reanalyzed data from a longitudinal cohort study of leptospirosis in an endemic urban slum setting. We first estimated the titer decay rate using historical data from a well-characterized point-source outbreak of leptospirosis with no reexposure (10). We then applied this decay rate to the cohort data. However, instead of directly comparing observed titers, we defined infections by comparing the second titer with a decayed version of the first. Although we focused on leptospirosis, the methods developed are readily adaptable to other infectious diseases.

METHODS

Estimating the rate of *Leptospira* antibody titer decay

There are numerous seroincidence studies of human leptospirosis (10, 20–27), but we identified only one that conducted longitudinal follow-up where reexposure was unlikely. This study, by Lupidi et al. (10), followed 18 patients who experienced clinical disease after a point-source outbreak of leptospirosis caused by a dead hedgehog that contaminated an Italian town's drinking water in 1984. These patients were followed longitudinally, with samples taken "by the third or fourth week after the onset of disease" and at 9, 18, 36, and 54 months after infection. The outbreak serovar was identified as a member of serogroup Australis but was not conclusively identified, so the authors reported data for 3 serovars within this serogroup. At the time of the outbreak, seroprevalence rates for Australis serovars in this area of Italy were low (28), and seroprevalent cases were epidemiologically distinct from outbreak cases (28, 29), so reexposure among outbreak cases was unlikely.

Samples were tested using the microscopic agglutination test (MAT), the gold standard serodiagnostic test for leptospirosis (30). The MAT is conducted by combining serially diluted patient serum samples with a standard amount of a *Leptospira* reference strain, and then examining the mixture under darkfield microscopy. The cutoff for a positive result is 50% agglutination of the bacteria by the patient sample, and the result is given as the reciprocal of the highest dilution at which this endpoint is reached. This procedure is repeated for an epidemiologically relevant battery of reference strains. Dilutions used in this study were 1:50, 1:100, 1:320, 1:1,000, 1:3,200, 1:10,000, and 1:32,000. Data were extracted from Figure 1 of the published report (10).

To model titer decay, we first defined an unobservable variable W to be a patient's true antibody level at the time of measurement. The result of a dilution assay like the MAT is an observed number of dilutions $K = 0, 1, 2,$ etc., at which the sample meets the agglutination threshold. These dilutions represent an interval-censored version of W. Specifically, the observed dilution $K = k$ is equivalent to $d_k < W < d_{k+1}$, where the d_k are known constants based on the dilution factor. We then specified a model in which the expected value of W at time t since infection is $m(t) = at^{\gamma}\exp(-\beta t)$. The constant a is a scaling factor that varies between individuals and serovars to account for heterogeneity in antibody decay (31–33). The parameter γ allows for an initial increase in antibody response postinfection

if $\gamma > 0$. The parameter β represents the rate of exponential decay in antibody response following the initial rise, if any (34, 35); for example, when $\gamma = 0$, a value $\beta = 0.2$ would indicate that the expected response at time $t + 1$ is $\exp(-0.2) = 0.819$ times the expected antibody response at time t. Additional technical details of the model and model-fitting are given in Web Appendix 1 (available at https://doi.org/10.1093/aje/kwaa253).

Effect of accounting for *Leptospira* titer decay in longitudinal cohort data

We then applied the estimated decay rate to data from an ongoing longitudinal cohort study in an urban slum in Salvador, the third largest city in Brazil. A previous, annually sampled cohort in this site had a crude seroincidence rate of 37.8 infections per 1,000 person-years (18) and a reinfection rate of 71.7 per 1,000, indicating frequent reexposure. This study also identified the peridomestic environment as a high-risk setting for leptospirosis (18, 19, 36). To better understand leptospiral dynamics and risk factors, we conducted a second longitudinal cohort study in which participants were sampled every 6 months (37). To be eligible, residents had to sleep at least 3 nights per week in the study site, be at least 5 years old, and consent to participate. This study was approved by the Yale University Ethics Committee (protocol 1,006) and Brazilian National Committee for Ethics in Research (protocol 963/2008). All participants provided written informed consent. Consent was also obtained from parents or guardians of participants who were minors. The study enrolled 2,421 of 3,716 eligible residents (37). We used the first 5 biannual samples, taken in 2013–2015, to quantify the effect of accounting for titer decay. We analyzed individuals with complete follow-up for each of the four 6-month and two 12-month intervals during this period. Table 1 shows the number of individuals for each interval and the corresponding seroincidence rates. In this study, samples were analyzed using successive 2-fold MAT dilutions, 1:50, 1:100, 1:200, . . . , 1:12,800. We restricted our analysis to the single serovar that causes 90% of infections in this setting (*L. interrogans* serovar Copenhageni) (19).

We used multiple imputation to apply titer decay to the cohort data. We first imputed a value for the titer at the beginning of a follow-up interval (W_1) using a uniform distribution on the log-scale. For example, an observed number of dilutions $K_1 = 2$, corresponding to a titer of 1:100, was randomly assigned a value of W_1 in the interval between 2 (inclusive) and 3 (exclusive). Colleagues with extensive MAT experience did not feel there was evidence to justify a nonuniform imputation distribution. We then applied the estimated decay rate over the interval between the samples to generate an expected titer at the end of the interval (W_1^*), using the formula $W_1^* = W_1 \times R^u$, where $R = \exp(-\beta)$ and u is the intersample interval in months. We then recensored W_1^* to obtain an imputed number of dilutions K_1^* by rounding down to the nearest whole number. This reflects the MAT procedure in which samples that do not meet the threshold at a given dilution are assigned a lower titer, effectively rounding them down. Finally, we defined infections by comparing K_2 with K_1^* instead of the conventional K_2 to K_1. As an illustrative example, consider an individual with $K_1 = 3$ (a titer of 1:200), and $K_2 = 4$ (1:400) taken 6 months apart. Conventional interpretations would not define an infection in the intersample interval. Our method first imputes a value of W_1 between 3 (inclusive) and 4 (exclusive), for example 3.25. We then calculate $W_1^* = W_1 \times R^u = 3.25 \times 0.926^6 = 2.05$. This value of K_1^* is rounded down to 2 (a titer of 1:100) and compared with the K_2 of 4, resulting in an infection defined using the

Table 1. Comparison of Infections Defined Using Conventional and Modified Interpretations of Paired Sera Among Participants in a Longitudinal Cohort Study Conducted in Pau da Lima, Salvador, Brazil, 2013–2015

Interval	Complete Follow-ups	Conventional Interpretation			Accounting for Titer Decay[a]		
		SC	FFT	Infection Rate[b]	SC Median (IQR)	FFT Median (IQR)	Infection Rate[b] Median (IQR)
6 months							
Period 1	1,593	48	16	40.2	58 (57, 60)	14 (13, 15)	45.8 (45.2, 46.5)
Period 2	1,485	87	9	64.6	99 (98, 101)	7 (6, 8)	71.4 (70.7, 72.7)
Period 3	1,601	36	11	29.4	43 (42, 44)	21 (20, 22)	40.0 (39.4, 40.6)
Period 4	1,399	52	14	47.2	68 (66, 70)	19 (17, 20)	62.2 (60.8, 62.9)
12 months							
Year 1[c]	1,444	69	3	49.9	109 (107, 110)	11 (10, 11)	82.4 (81.7, 83.1)
Year 2[d]	1,292	36	5	31.7	64 (61, 67)	20 (20, 21)	65.8 (63.5, 67.3)

Abbreviations: IQR, interquartile range; FFT, 4-fold titer rise; SC, seroconversion.
[a] The values for the interpretation allowing for titer decay are based on 10,000 imputations using a decay rate sampled from its distribution.
[b] Per 1,000 population.
[c] Periods 1 and 2.
[d] Periods 3 and 4.

4-fold rise criterion allowing for titer decay. See Web Figure 1 for a visual example. We repeated this procedure 10,000 times to estimate variation due to the imputation process. As a sensitivity analysis, we also repeated the procedure 10,000 times sampling the decay rate from the sampling distribution of its estimate instead of using the point estimate R.

Two concerns need to be mentioned. One is that titer kinetics in clinical cases in a naive population (Lupidi et al. (10)) might differ from those in a leptospirosis-endemic setting where individuals might have some level of immunity that would affect their titer decay rate (14, 38). We took 2 steps to ensure that applying the estimated decay rate to the cohort data was valid. First, we sampled 16 cohort participants within a 6-month interval, creating intervals of approximately 4, then 2, months. We visually compared the titer kinetics of these individuals with those of the individuals in Lupidi et al. (10). Second, to examine the sensitivity of our results to the decay rate, we repeated the above procedure using decay rates both faster and slower than the one estimated from the Lupidi et al. data. The decay constant was calculated by $1 - (1 - R)$. To calculate alternative decay rates, we added a multiplier M to the equation: $1 - (1 - R)M$. We used $M = 2$ (to calculate a decay rate twice as fast as estimated), 1/2, and 1/4 (to estimate decay rates one-half and one-quarter as fast as estimated, respectively). The second concern is whether titer kinetics are similar when different *Leptospira* serovars cause infections. This cannot currently be addressed because no comparable longitudinal data without reexposure exist for *L. interrogans* serovar Copenhageni.

RESULTS

MAT titers from Lupidi et al. (10) varied by serovar and individual and were well modeled by exponential decay (Web Table 1 and Web Figure 2). The 3 serovars had significantly different intercepts, with the highest for serovar Lora, then Bratislava, then Australis (see Web Table 2 for parameter estimates). Our final model of exponential decay over time in months, with an intercept affected by serovar and individual, had an estimated decay rate R of 0.926 dilutions per month (95% confidence interval: 0.918, 0.934).

The parameter representing the initial titer rise was not statistically significant, so we excluded it (Web Table 1).

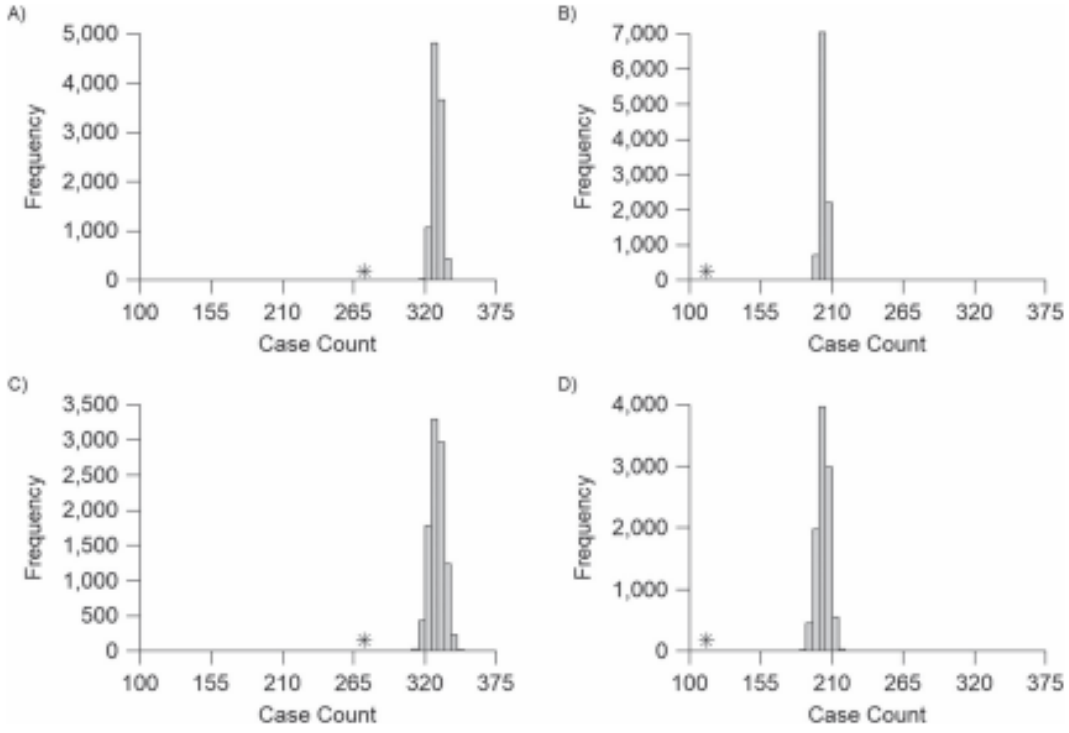

Figure 1. Comparison of infections defined using conventional and modified interpretations of paired serology among participants in a longitudinal cohort study conducted in Pau da Lima, Brazil, 2013–2015. In each panel, the case count using conventional interpretations of paired serology, which do not account for titer decay, is marked by an asterisk. Histograms show the number of infections defined in each of 10,000 imputations allowing for titer decay over all 6-month periods (A, C) or all 12-month periods (B, D). A and B use the point estimate of the decay rate, and C and D use a decay rate sampled from the distribution of the estimate.

Table 2. Sensitivity Analysis Demonstrating the Effect of Decay Rate on the Number of Infections Defined Using Paired Serology Among Participants in a Longitudinal Cohort Study Conducted in Pau da Lima, Brazil, 2013–2015

Titer Decay Rate	Total Infections[a] Per 6-Month Period, Median (IQR)			
	Period 1	Period 2	Period 3	Period 4
Conventional (no decay)	64	96	47	66
0.981 (*R*/4)	66 (65–67)	98 (97–99)	51 (50–53)	69 (68–71)
0.962 (*R*/2)	69 (68–70)	100 (99–101)	56 (55–58)	73 (72–75)
0.926 (*R*)	73 (72–74)	106 (105–108)	64 (63–65)	87 (85–88)
0.857 (2*R*)	82 (81–83)	120 (120–121)	77 (76–78)	101 (100–101)

Abbreviations: IQR, interquartile range; *R*, estimated decay rate.
[a] Total infections consists of seroconversions plus 4-fold rises.

Our inability to characterize initial titer rise in the Lupidi et al. (10) data is likely due to the lack of samples during the first month after infection, when the rise would occur. We attempted to resolve the initial titer rise by imputing the missing time of the first sample for each individual based on their titer trajectory, but this did not increase the precision of our estimates. The fixed 6-month periods between serosurveys in the Pau da Lima cohort also prevented us from characterizing initial titer rise from that data.

There was no visual evidence that titer decay in participants in the Brazilian cohort differed significantly from that of cases in Lupidi et al. (10) (Web Figure 3), so we applied the estimated decay rate to the longitudinal cohort data. Summaries of infections defined using both conventional interpretations and allowing for titer decay are presented in Table 1. Infections are reported for each of the four 6-month intervals as well as the two 12-month intervals. The imputation variance—captured by the interquartile range of infection counts across the 10,000 iterations—was small, indicating that each imputation produces comparable results. Results are reported for the analysis using a titer decay rate sampled from its distribution. Results using the decay rate point estimate are qualitatively similar, with narrower confidence intervals (Figure 1).

Accounting for titer decay increased the number of infections. While the mean 6-month infection rate under the conventional definition was 45.3 infections per 1,000 follow-ups, when allowing for decay the mean rate became 54.8/1,000. The difference was even more pronounced when considering 12-month sampling intervals, for which the mean infection rate went from 40.8/1,000 to 74.3/1,000 when accounting for titer decay (calculated from Table 1 values). Sensitivity analysis showed that even a decay rate only one-fourth as fast as that estimated from the Lupidi et al. data (10) would still result in more infections than the conventional definition (Table 2).

DISCUSSION

We used likelihood-based estimation to model the decay of interval-censored leptospirosis antibody titers, and then applied that decay rate to longitudinal cohort data to more accurately measure seroincidence. Our model of leptospiral MAT titer decay demonstrated that accounting for decay substantially increased infection rates. We took steps to ensure that our decay estimate was reasonable but showed that even accounting for a considerably slower-than-estimated decay rate identified more infections than the conventional interpretation.

Our findings have important implications for epidemiologic study design and interpretation. The biannual incidence rate of leptospirosis in our study site was 1.21 times the rate estimated by conventional interpretations, representing a substantial increase in burden. We also identified that the intersample interval has important implications for interpretation. Leptospirosis incidence was underestimated by conventional definitions with both 6- and 12-month intervals, but the effect was much stronger with the longer interval. In our case, the estimated annual incidence rate was 1.82 times the value calculated with conventional interpretations. This calls into question the validity of direct comparisons among longitudinal studies with different sampling intervals. For other pathogens, the validity of such comparisons depends on the relative timescales of antibody decay and intersample intervals. Longitudinal cohort studies aim not only to calculate burden but also to determine risk factors for infection, and these previously unidentified infections could modify the risk factors identified from our data (18, 19, 36). Accurate burden and risk factor information is critical for public health practitioners working to prevent infection as well as researchers working to understand infection dynamics and trends. In addition, improved understanding of individual sequences of infection events could help evaluate whether preexisting antibody titers lower the risk of a subsequent infection. This has important implications for vaccine development (39). Finally, cohort study design could be influenced by these results, because a higher incidence rate will lower sample-size requirements.

Our method for quantifying decay and seroincidence has several benefits. The modeling framework is relatively simple and can be applied to other data sets and pathogens with minimal modifications. Beyond the paired samples to

be analyzed, the method requires a single data set without reexposure to estimate the titer decay rate. While more data sets would increase the accuracy and generalizability of the estimated decay rate, we demonstrated that even a single data set can provide useful information. In addition, our method preserves the 4-fold rise criterion to avoid the misclassification possible when using a 2-fold rise as the infection standard at the individual level (1). Finally, our method takes into account the actual time elapsed between samples, making it flexible to variation in the intersample interval both within and between studies.

While we applied this method to leptospirosis, it is suitable for application to other infections. For example, brucellosis (40) and scrub typhus (a rickettsial infection) (41) are underreported infections commonly diagnosed using paired serology. If antibody decay rates can be calculated in the absence of reexposure, those rates can then be applied to longitudinal cohort studies to generate more accurate burden measures. However, data sets without reexposure can be lacking for endemic infections with a high force of infection. Another consideration when applying this method is the number of strains circulating. In our system, a single leptospirosis serovar causes most infections (19, 37). In areas where a single dengue virus serovar circulates, our method would apply. However, in the presence of multistrain dynamics, including cross-reactivity and -immunity—common for dengue virus and influenza—modifications would be required. In these more complex situations, models account for these dynamics by simultaneous estimation of titer increase, titer decay, and infection (8, 9, 11, 13, 14). To eliminate potential statistical identifiability issues (15) and simplify these models, our titer decay model could be incorporated as a deterministic process.

This study has the following limitations. The MAT is subjective and results vary by performer and laboratory. However, this is a common feature of dilution assays. Our method mimics the MAT procedure and accounts for some variability through the imputation process. We used a single historical data set to fit our model. To our knowledge, this is the only published data set of longitudinal human MAT follow-up in which reexposure is unlikely. Reexposure cannot be conclusively ruled out, but the low seroprevalence rate to outbreak strains (28), epidemiologic differences between outbreak and seroprevalent cases (28, 29), and lack of increased titers during longitudinal follow-up of outbreak cases (10) all make reexposure improbable. Individual starting titers varied, potentially due to differences in immune factors or the infecting dose. Separating these 2 factors is a major challenge in serological studies and one we cannot overcome with this data. The small data set ($n = 18$) limited us to considering a relatively simple exponential decay model. In particular, the timing of the samples limited our ability to account for any noninstantaneous initial rise in titer following infection.

Interval-censored serological assays are used to define infection with a range of pathogens (1, 9, 30), but we demonstrated that conventional interpretations of paired sera with long intersample intervals can substantially underestimate infection rates. Titer decay is therefore an important phenomenon to account for when interpreting paired serology conducted with long intersample intervals. Modeling the decay of interval-censored titers in the absence of reexposure allowed us to estimate a titer decay rate that we used to understand transmission in a more complex setting. Our method is flexible and generalizable to other infectious diseases defined using interval-censored assays.

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O artigo 3, “**Biannual and quarterly comparison analysis of antibody kinetics on a subcohort of individuals exposed to *Leptospira* interrogans in Salvador, Brazil”**, foi publicado na revista Frontiers in Medicine, section Infectious Diseases – Surveillance, Prevention and Treatment (DOI: 10.3389/fmed. 2022.862378). Neste artigo, avaliamos a cinética de anticorpos em indivíduos de uma área endêmica para leptospirose em Salvador, Brasil, comparando dois tempos diferentes de coleta entre as amostras.

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Biannual and Quarterly Comparison Analysis of Agglutinating Antibody Kinetics on a Subcohort of Individuals Exposed to *Leptospira interrogans* in Salvador, Brazil

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Introduction: Leptospirosis is a zoonosis with a worldwide spread that leads to clinical manifestations ranging from asymptomatic infection to a life-threatening disease. The immune response is predominantly humoral mediated limited to the infecting serovar. Individuals living in an area endemic for leptospirosis are often exposed to an environment contaminated with leptospires and there is a paucity of information on naturally acquired immunity. In the present study, we evaluated the kinetics of agglutinating antibodies in individuals from an endemic area for leptospirosis in Salvador, Brazil comparing two different intersample collection times.

Methods: Between 2017–2018, we carried out a biannual prospective cohort with 2,086 individuals living in an endemic area for leptospirosis in Salvador, Brazil. To compare agglutinating antibody kinetics using microscopic agglutination test (MAT) with different collection times, a subcohort of 72 individuals with quarterly follow-up was carried out in parallel.

Results: The results revealed that using a shorter time for intersample collection led to the detection of a higher number of infections and reinfection events. Furthermore, we observed a higher rate of titer decay indicating partial and short protection. However, there was no indication of major changes in risk factors for the disease.

Conclusions: We evaluated antibody kinetics among residents of an endemic area for leptospirosis comparing two sample collection times. The constant exposure to the contaminated environment increases the risk for leptospirosis infection with reinfection events being more common than expected. This indicates that the burden of leptospirosis might be underestimated by serological surveys, and further studies are necessary to better characterize the humoral response after infection.

Keywords: *Leptospira*, leptospirosis, human, serosurvey, MAT, antibody kinetics

INTRODUCTION

Leptospirosis is a zoonosis of worldwide distribution and an important reemerging disease caused by pathogenic spirochetes of the genus *Leptospira* (1). The disease is endemic in a diverse range of epidemiological settings given the high number of animal reservoirs that can harbor the bacteria in their kidneys and excrete in their urine (2, 3). The transmission in humans occurs mainly through contact with environmental sources contaminated with the urine of infected animals. Rodents are the main source of human infection and responsible for the maintenance of the bacteria in the urban environment (4, 5). The clinical manifestations of the disease vary from asymptomatic or mild to severe disease such as Weil syndrome and pulmonary hemorrhage syndrome, associated with a lethality rate of 10 and 50%, respectively (3, 6–8).

The World Health Organization (WHO) estimates the occurrence of more than one million human cases of leptospirosis worldwide, with more than 50,000 deaths each year, most of which occurs in developing countries and tropical and subtropical climate regions (9, 10). In Brazil, 13,000 severe cases are reported per year with a lethality rate of 10.8% (11). The occurrence of urban epidemics, in Brazil and similar regions, is associated with environmental, occupational, and recreational risk factors and the large population of reservoirs (4, 12). More than 300 serovars can cause disease in humans and animals (13), but serovars from *L. interrogans* species are the most pathogenic and common throughout the world (14). In Brazil, serovar Copenhageni is the leading cause of epidemics in urban environments representing more than 90% of infections in Salvador with *Rattus norvegicus* (rat or sewer rat) as the main carrier (11, 15).

During the course of leptospirosis infection, the immune response is predominantly humoral mediated by the production of circulating antibodies directed against the lipopolysaccharide (LPS) and limited to the infecting serovar (16). Individuals living in regions where leptospirosis is endemic, are frequently being exposed to *Leptospira* and there are reports of the presence of anti-*Leptospira* antibodies in patients recovering from severe disease and in individuals with no previous history of the disease, most likely resulting from asymptomatic infection (17). However, there is little information on whether these individuals develop a naturally acquired protective immunity against infection or severe disease (17). In the present study we evaluated the kinetics of the humoral response after leptospirosis infection on individuals living in an urban slum area to obtain relevant information that may contribute to the better understanding of the naturally acquired immunity against leptospirosis reinfection.

MATERIALS AND METHODS

Ethics Statement

This project was approved by the Research Ethics Committee of the Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), the National Research Ethics Council (CONEP) through the Certificate of Presentation for Ethical Appreciation (CAAE) #45217415.4.0000.0040 and the Yale University Institutional Review Board #1006006956. All participants received guidance on the objectives, procedures, and risks associated with participation during informed consent. Minors gave verbal consent and we obtained written consent by their parents or legal guardian. Collegiality of participation was assured. Laboratory results were made available to the cohort participants.

Study Site and Population

A prospective cohort study was carried out in the community of Pau da Lima (13° 32′53.47 "S: 38° 43′51.10" W), an urban slum community of Salvador, Bahia, Brazil (**Figure 1**). This community was described as an area of 0.24 km^2 made up of three valleys, with open sewage close to the residences, places of garbage accumulation, and risk of flooding mainly in the bottom areas of the valleys (**Figure 1**). Due to the irregular occupation of the land and the precarious urban infrastructure, the community of Pau da Lima presents similar conditions to other vulnerable communities in Brazil and tropical regions of the world (9, 18). Previous studies have shown that residents of this community are in contact with the contaminated environment throughout the year leading to a high risk of leptospirosis infection facilitated by rat infestation, contact with mud promoted by topographic factors such as home elevation and inadequate drainage (9, 19). The crude rate of *Leptospira* infection in this community was 37.8 per 1,000 person-years with a 2.3-fold higher rate of secondary infection when compared to the rate of primary infection (19).

Between September 2017 to December 2018, residents of Pau da Lima with ≥5 years and who slept at least three nights a week at home were enrolled in the biannual analysis study. During all visits (every 6 months), blood samples were collected for evaluation of anti-leptospire antibodies using the microscopic agglutination test (MAT). Previously validated epidemiological, exposure and sociodemographic questionnaires were applied annually for field data collection. At the moment of enrollment for the biannual study, individuals with a previous history of leptospirosis infection determined by MAT were enrolled for our subcohort, together with controls that had no history of infection. For this subcohort, a quarterly analysis follow-up was performed (every 3 months) for blood collection, with a total of five home visits to assess antibody kinetics. For the entire study, we considered as exclusion criteria the following: participant not found at the time of the team visit, refusal to participate in the study and inability to locate the participants after three attempts.

Serologic Evaluation for *Leptospira* Infection

The blood samples collected were sent to the Laboratory of Pathology and Molecular Biology at Instituto Gonçalo Moniz, Fiocruz, BA. Sera was obtained through centrifugation, and it was processed for the presence of agglutinating antibodies against *Leptospira* using the microscopic agglutination test (MAT). The MAT was performed with *L. interrogans* serovar Copenhageni strain Fiocruz L1-130, the most prevalent serovar in the region (9, 15, 18, 19). The screening was performed using 1:50 and 1:100 dilutions of serum. A positive sample was determined when agglutination was observed on more than 50% of leptospires compared to the control with no sera. If the sera were positive at the dilution of 1:100, the sample was titrated to

FIGURE 1 | Slum community site in the city of Salvador, Brazil. **(A)** Aerial photograph showing the study site boundary in the community of Pau da Lima, Brazil. **(B)** Topographic map highlighting the households participating in the subcohort study. In blue, households with uninfected individuals; in yellow, households with infected individuals and in red, households with reinfected individuals. Photographs showing social characteristics **(C)** and environmental risk factors **(D)** of the community.

determine the highest agglutination titer. The presence of anti-*Leptospira* agglutinating antibodies was used as a marker for previous infection. A case of *Leptospira* infection was defined as individuals with antibody titers ≥1:50 at any time-point, and/or with a seroconversion between consecutive time-points, defined as the absence of agglutination reaction in the first sample and the presence of agglutination with ≥1:50 in the following sample, and/or four-fold rise in titer between consecutive time-points. Reinfection was defined as participants who had two or more infections documented based on the MAT results during the follow-up period.

Statistical Analyses

Statistical analyses were performed using the RStudio package, version 1.2.5033. Descriptive analysis was performed to obtain absolute frequencies or means and medians for categorical variables and univariate analysis through Welch's two-sample *t*-test and Pearson's chi-square test, with 95% CI. Statistical significance was considered when the probability value $p \leq 0.05$. GraphPad Prism version 7 for Windows was used to evaluate the kappa coefficient of agreement between collection times and the kinetic data calculated in log10 and plotted against collection periods. A logistic regression model was used to determine the adjusted odds ratio (OR) (95% confidence intervals) to assess whether MAT titers protect against reinfection.

RESULTS

Enrollment and Follow-Up of Study Participants

During a cohort study for leptospirosis conducted from 2017–2018, a total of 2,086 individuals were enrolled for a biannual collection of blood for MAT assay and analysis of epidemiological data. Among them, 339 (16%) were serologically confirmed for leptospirosis infection. We enrolled 110 participants for a subcohort performing a quarterly analysis follow-up. Among those, 52 confirmed cases and 20 negative cases that had ≥4 blood samples at the end of the study were analyzed (**Figure 2**).

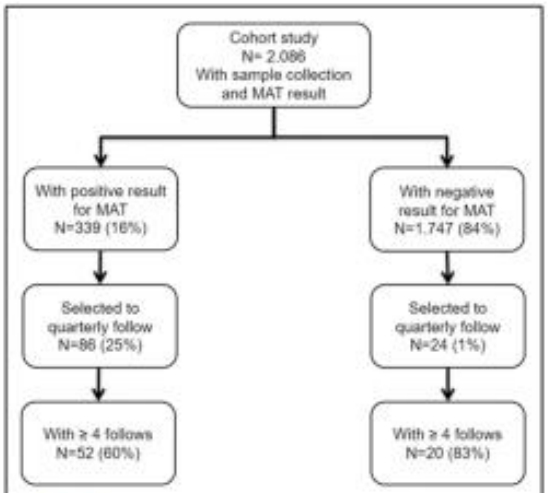

FIGURE 2 | Flowchart of the study participants enrollment.

Characteristic of the Participants

The demographic, socioeconomic and exposure information of all the cohort participants and the subcohort are shown

TABLE 1 | Sociodemographic and exposure characteristics of the participants enrolled in the study.

Characteristic	Cohort total (*N* = 2,086)[a]	Subcohort (*N* = 72)[a]
Median Age (years)	27 (17)	28 (16)
Age (years)		
05–14	631 (30.2%)	21 (29.2%)
15–24	404 (19.4%)	9 (12.5%)
25–34	399 (19.1%)	18 (25.0%)
35–44	292 (14.0%)	12 (16.7%)
> 44	360 (17.3%)	12 (16.7%)
Sex		
Female	1,206 (57.8%)	36 (50.0%)
Male	880 (42.2%)	36 (50.0%)
Ethnicity		
Black	944 (45.3%)	29 (40.3%)
Brown	969 (46.5%)	33 (45.8%)
White	154 (7.4%)	10 (13.9%)
Others	19 (0.9%)	0 (0.0%)
Education		
Up to 9th year	1,632 (78.2%)	56 (77.8%)
More than 9th year	454 (21.8%)	16 (22.2%)
Married or stable union	730 (35.0%)	22 (30.6%)
Informal employment	763 (36.6%)	36 (50.0%)
Per capita household income (US$/day)	4.7 (4.8)	4.3 (3.7)
Cleaned sewage	311 (14.9%)	10 (13.9%)
Open sewage at <10 m from home	1,464 (70.2%)	50 (69.4%)
Accumulated trash within <10 m of home	781 (37.4%)	24 (33.3%)
Sewage contact	777 (37.2%)	33 (45.8%)
Floodwater near home	822 (39.4%)	40 (55.6%)
Mud near home	949 (45.5%)	45 (62.5%)
Work in construction	179 (8.6%)	5 (6.9%)
Work related to hawker	47 (2.3%)	3 (4.2%)
Work related to garbage removal	74 (3.5%)	7 (9.7%)
Work involves contact with mud	60 (2.9%)	3 (4.2%)
Work involves contact with flood water	50 (2.4%)	3 (4.2%)
Work involves sewage contact	44 (2.1%)	3 (4.2%)
Fever	559 (26.8%)	16 (22.2%)

[a] *Median (IQR); n (%).*

in **Table 1**. Most of the participants were young adults with a median age between 27–28 years old, with 57.8% of women for the cohort and 50% for the subcohort, respectively. Regarding ethnicity, there was a predominance of brown race (46.5% for the cohort and 45.8% for the subcohort) and <9 years of education was reported in all groups (78.2 and 77.8%, respectively). Informal employment was described by 36.6% of the cohort and 50% of the subcohort participants. Construction work (8.6% for the cohort and 6.9% for the subcohort) and work related to garbage removal (3.5 and 9.7%, respectively) were most described activities in the groups. The median per capita household income was similar among groups, with US$ 4.7 daily for the cohort and US$ 4.3 daily for the subcohort. Among the exposure variables, in all groups open sewage was reported

TABLE 2 | Concordance between the biannual and quarterly follow-up collections.

Quarterly follow-up	Biannual follow-up			
	Infection	Reinfection	No infection	Total
Infection	12	2	8	22
Reinfection	13	10	2	25
No infection	0	0	25	25
Total	25	12	35	72

< 10 m from the house (70.2 and 69.4%), contact with sewage (37.2 and 45.8%), contact with floodwater near home (39.4 and 55.6%), contact with mud near home (45.5 and 62.5%) and 14.9% of the total cohort reported cleaning sewage followed by 13.9% of the subcohort group.

The kappa statistics for leptospirosis case classification at the different collection times was 0.48 (95% CI: 0.32–0.63), achieving moderate agreement (**Table 2**). In this evaluation we identified differences in leptospirosis case classifications when comparing biannual analysis and quarterly analysis collections, mainly between the infected vs. reinfected and no-infected vs. infected groups. When performing a biannual analysis, we identified 25 (34.7%) infections, 12 (16.6%) reinfections and 35 (48.6%) negative individuals, while in the quarterly analysis, we identified 22 (30.5%) infections, and 25 (34.7%) reinfections and non-infections, each. There are 13 (18%) individuals that would be classified as reinfection rather than infection when performing a quarterly analysis (**Table 2**, **Figure 3**). Furthermore, the quarterly analysis identified an extra 8 (11%) individuals as infection and 2 (2.8%) individuals as reinfection rather than no-infection determined by the biannual analysis (**Table 2**, **Supplementary Figure S1**). In contrast, there were only 2 individuals classified as reinfection by the biannual analysis that would be considered as infection by the quarterly evaluation (**Table 2**, **Supplementary Figure S1**). The multivariate analysis did not find an association between MAT titers and reinfection (**Table 3**). Taken together, those results indicate that the decay of agglutinating antibodies is shorter than expected in individuals exposed to leptospires without severe symptoms, and the time between assessment of those antibodies can influence the number of infections and reinfections. Further, our data suggests that agglutinating antibodies might not be the ideal correlates for naturally acquired immunity against reinfection.

Agreement Analysis

The distribution of MAT titers of samples positive for the Fiocruz L1-130 strain and their frequencies are listed in **Supplementary Table S1**. Of the total, 47 (65%) had anti-leptospire agglutinins in the quarterly analysis collection while 37 (51%) in the biannual analysis collection. Most participants in the group with quarterly analysis collection had low titers, with 62% ranging from 1:50 (28%) to 1:200 (21%). The highest agglutination titers in the biannual analysis collection period were observed at the 1:400 dilution (41%). Those results indicate that most of the exposures lead to low agglutinating titers.

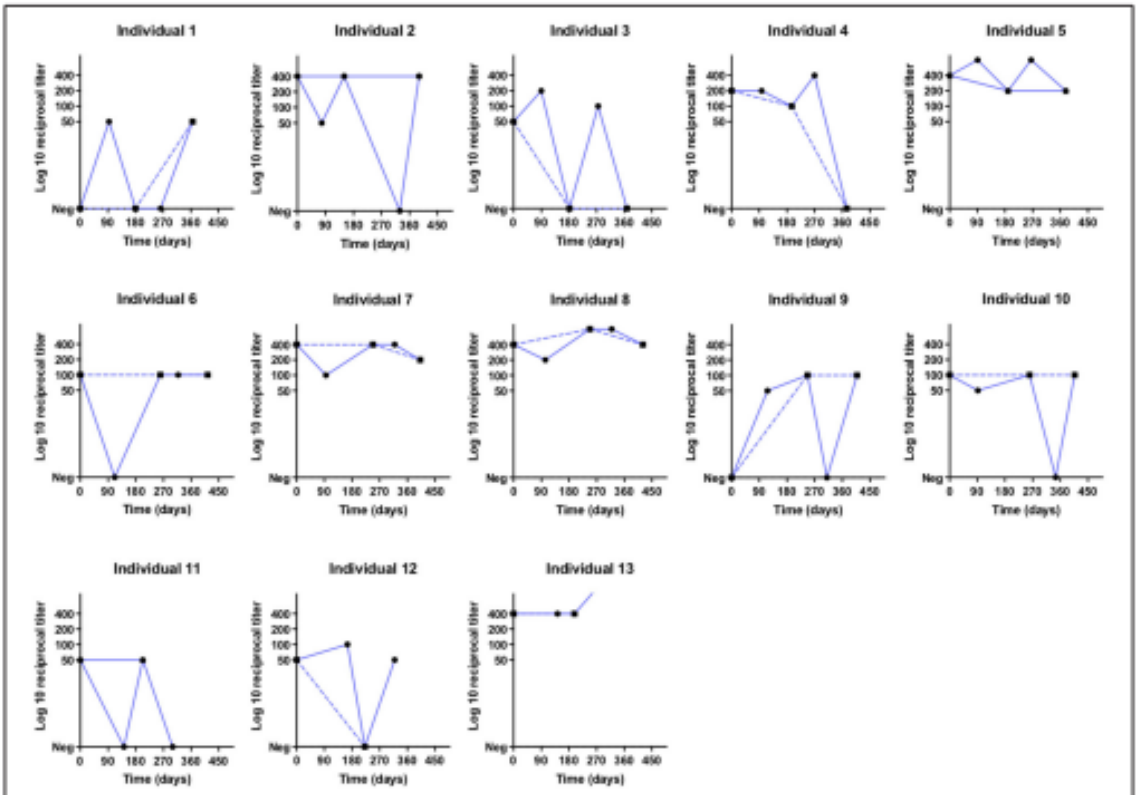

FIGURE 3 | Titration curves in log10 of individuals classified as reinfection in the quarterly analysis (•) and infection in the biannual analysis (▲) at different times (days) of collection.

Biannual vs. Quarterly Follow-Up Analysis

We then evaluated the characteristics of the subcohort participants stratified by infection and non-infection based on different collection times (biannual vs. quarterly analysis) (**Table 4**). The variables age ($p < 0.001$ and $p = 0.005$) and cleaning sewage ($p = 0.003$ and $p = 0.033$) were associated with a higher chance of infection for both groups with different collection times. Furthermore, informal employment ($p = 0.013$) for the quarterly analysis collection group and being married or having a stable union ($p = 0.032$) for the biannual were also associated with a higher chance of infection when compared to the non-infection group (**Table 4**). A more detailed analysis of the participants per collection time stratifying the infection group by single infection and reinfection event showed that cleaning sewage was the only significant risk for infection ($p = 0.04$) on the quarterly analysis group (**Supplementary Table S2**). For reinfection, age ($p = 0.03$) and informal employment ($p = 0.01$) were both associated with a higher risk (**Supplementary Table S2**). A similar analysis using the biannual analysis data showed that variables age and cleaning sewage were both significant risks for infection and reinfection (**Supplementary Table S3**). Interestingly, having a married or stable union ($p = 0.047$) showed as a risk for infection on the biannual analysis (**Supplementary Table S3**). There was no statistically significant difference when comparing the infection and reinfection groups in both analyses with different collection times. Those results showed that despite the differences of infections and reinfections rates among the two different analyses, the risk for overall infection is similar in both groups, indicating that the time between serological evaluation might not have a major impact on the outcome of risk analysis.

DISCUSSION

The immunity against leptospirosis is based on a short-term humoral response for humans and animals (14). However, the few existing studies that report the kinetics of antibodies were performed in clinical patients associated with disease severity (20, 21) or in experimental animal models (14, 22). Evaluating antibody kinetics in individuals with natural *Leptospira* infection will help to better understand the duration of the immune response after infection, the course of infection, and the dynamics

TABLE 3 | Multivariate analysis to evaluate MAT titers as immune markers for reinfection on biannual and quarterly follow-up.

	Biannual analysis ($N = 47$)			Quarterly analysis ($N = 47$)		
Characteristic	**OR[a]**	**95% CI[b]**	***p*-value**	**OR[a]**	**95% CI[b]**	***p*-value**
(Intercept)	0.96	0.33 – 2.80	0.941	0.82	0.29 – 2.26	0.701
Sewage contact						
No	—	—		—	—	
Yes	1.08	0.33 – 3.54	0.894	1.26	0.37 – 4.43	0.713
Titers of MAT						
≤100	—	—		—	—	
≥200	2.33	0.49 – 13.35	0.303	4.26	0.52 – 90.63	0.226
400	0.8	0.16 – 3.94	0.786	1.69	0.45 – 6.72	0.438
≥800	1.01	0.19 – 5.44	0.991	0.34	0.02 – 3.21	0.386

[a] *OR = Odds Ratio.*
[b] *CI = Confidence Interval.*

of protective immunity. In this study, we had the opportunity to evaluate the kinetics of the antibody response and the factors associated with exposure to leptospirosis in naturally infected individuals with asymptomatic infection comparing a biannual and a quarterly serological analysis. Among the 72 individuals who participated in the subcohort with ≥4 quarterly collections, we found that 65% had circulating anti-*Leptospira* antibodies. The variables age ($p < 0.001$ and $p = 0.005$) and cleaning sewage ($p = 0.003$ and $p = 0.033$) were associated with a higher chance of infection in both analyses.

The time of blood collection between samples can affect the number of infection and reinfection of leptospirosis. A recent study applied a titer decay rate on the serological data of the same population in Salvador, Brazil and identified a higher number of mean infection rate on the biannual analysis and even higher when applying the decay to annual analysis (23). In agreement with this report, our quarterly serological analysis identified a higher number of leptospirosis infections and reinfections events in our population when compared with a biannual analysis, suggesting that exposures to the leptospirosis pathogen in this urban slum setting are frequent if not ubiquitous. Given the constant high risk for exposure to the pathogen observed in this community either by the environment (9, 18) or by the high mobility of its inhabitants (24) and the bias that a reexposure and potential boost of titers can do to titers decay (23), it might be impossible to calculate an accurate titer decay. This limitation can affect the correct incidence rate, data comparison among different longitudinal cohort studies and potentially risk assessment for exposure. Further considerations should be made on reducing the time of serological evaluation or applying decay rates estimations to take into account the differences observed here on titer decay.

Our results indicate that the humoral response detected by MAT is relatively short and provides partial protection against reinfection. Previous studies have reported that individuals with leptospirosis were protected against reinfection by the same *Leptospira* serovar or by related serovars for a short period (25, 26). However, a recent study from French Polynesia showed that individuals with a first infection might not be protected against subsequent reinfection (27). In our study, when performing a quarterly analysis, we observed that agglutinating antibodies have a short life span with titers up to 1:200 disappearing after 90 days. Also, our analysis showed that agglutinating antibodies don't seem to affect the risk for subsequent infection. These results are in agreement with previous data that showed that constant exposure and pre-existing anti-leptospire antibodies did not provide complete immunity (19). Of note, most of the titers observed in our quarterly analysis were low, with 62% ranging between 1:50 and 1:200. A recent study of an attenuated vaccine has shown that antibodies against proteins rather than agglutinating antibodies are correlated to protection (28). It is possible that individuals in this community, which are often being exposed to an environment contaminated with leptospires, have built an immune response similar to a live vaccine that reduces symptoms in case of reinfection and potentially providing cross-protection between unrelated *Leptospira* serovars (5, 29, 30). Further studies to better characterize the immune response after infection, focusing on B and T cell responses and memory, would provide valuable information about potential markers to protect against reinfection.

The time of sample collection and the higher infection rates don't seem to affect the major risk factors for leptospirosis infection. Despite the assumption that a more suitable analysis leading to higher infection rates could potentially affect the observed risk factors for the disease (23) our results indicate that regardless of the period of analysis the potential risks for infection are similar. Transmission dynamics and risk factors for *Leptospira* infection and reinfection are associated with environmental, demographic, and individual exposures. Our results show that risk factors for infection in this community corroborate previous studies (9, 18, 19). The chance of acquiring anti-leptospire antibodies was more frequent in young adults with <9 years of schooling, regardless of the time of collection. Although gender was not identified as a risk factor for acquiring infection in our study, several others consistently report that men in working age groups are at higher risk (9, 10, 15, 31, 32). In our study, being married or having a stable relationship was associated with a risk of infection. We also found that in this group 69% (11/16) of individuals with infection were women

TABLE 4 | Comparison of the sociodemographic and exposure characteristics of the individuals in the subcohort based on the biannual analysis and quarterly analysis follow-up.

Characteristic	Biannual ($N = 72$)			Quarterly ($N = 72$)		
	No infection ($N = 35$)[a]	Infection ($N = 37$)[a]	p-value[b]	No infection ($N = 25$)[a]	Infection ($N = 47$)[a]	p-value[b]
Age (years)			**<0.001**			**0, 005**
05–14	16 (45.7%)	5 (13.5%)		13 (52)	8 (17)	
15–24	4 (11.4%)	5 (13.5%)		2 (8.0)	7 (15)	
25–34	4 (11.4%)	14 (37.8%)		3 (12)	15 (32)	
35–44	2 (5.7%)	10 (27.0%)		1 (4.0)	11 (23)	
> 44	9 (25.7%)	3 (8.1%)		6 (24)	6 (13)	
Sex			>0.99			>0.99
Female	17 (48.6%)	19 (51.4%)		13 (52)	23 (49)	
Male	18 (51.4%)	18 (48.6%)		12 (48)	24 (51)	
Ethnicity			0, 34			0, 17
Black	13 (37.1%)	16 (43.2%)		8 (32)	21 (45)	
Brown	15 (42.9%)	18 (48.6%)		11 (44)	22 (47)	
White	7 (20.0%)	3 (8.1%)		6 (24)	4 (8.5)	
Others	0 (0.0%)	0 (0.0%)		0 (0)	0 (0)	
Education			0, 87			0, 53
Up to 9th year	28 (80.0%)	28 (75.7%)		21 (84)	35 (74)	
More than 9th year	7 (20.0%)	9 (24.3%)		4 (16)	12 (26)	
Married or stable union	6 (17.1%)	16 (43.2%)	**0, 032**	4 (16)	18 (38)	0, 092
Informal employment	14 (40.0%)	22 (59.5%)	0, 16	7 (28)	29 (62)	**0, 013**
Per capita household income (US$/day)	4.2 (4.1)	4.5 (3.3)	0, 67	3.9 (4.4)	4.6 (3.3)	0, 52
Cleaned sewage	0 (0.0%)	10 (27.0%)	**0, 003**	0 (0)	10 (21)	**0, 033**
Open sewage at <10 m from home	21 (60.0%)	29 (78.4%)	0, 15	15 (60)	35 (74)	0, 32
Accumulated trash within <10 m of home	11 (31.4%)	13 (35.1%)	0, 93	7 (28)	17 (36)	0, 66
Sewage contact	15 (42.9%)	18 (48.6%)	0, 8	12 (48)	21 (45)	0, 98
Floodwater near home	21 (60.0%)	19 (51.4%)	0, 62	15 (60)	25 (53)	0, 76
Mud near home	20 (57.1%)	25 (67.6%)	0, 5	15 (60)	30 (64)	0, 95
Work in construction	2 (5.7%)	3 (8.1%)	>0.99	1 (4.0)	4 (8.5)	0, 82
Work related to hawker	1 (2.9%)	2 (5.4%)	>0.99	1 (4.0)	2 (4.3)	>0.99
Work related to garbage removal	1 (2.9%)	6 (16.2%)	0, 13	1 (4.0)	6 (13)	0, 44
Work involves contact with mud	1 (2.9%)	2 (5.4%)	>0.99	1 (4.0)	2 (4.3)	>0.99
Work involves contact with flood water	1 (2.9%)	2 (5.4%)	>0.99	1 (4.0)	2 (4.3)	>0.99
Work involves sewage contact	1 (2.9%)	2 (5.4%)	>0.99	1 (4.0)	2 (4.3)	>0.99
Fever	5 (14.3%)	11 (29.7%)	0, 2	4 (16)	12 (26)	0, 53

[a] *Mean (SD) or Frequency (%).*
[b] *Welch Two Sample t-test; Pearson's Chi-squared test.*
The values in bold are the variables with statistical significance (≤ 0.05).

against 25% (4/16) of men. A study in Cali, Colombia, showed that the female gender was directly associated with the risk of *Leptospira* infection and that domestic factors may play an important role in transmission, particularly in urban slums (33, 34). Another possibility to explain this finding is related to factors such as age and exposure time. We observed that the general mean age in the group of married individuals with infection was 33 years, while for single individuals with infection it was 27 years. Our findings may be explained by the fact that being older resulted in longer exposure time and a greater risk of infection. Recently, a study on the same area showed that increasing age was associated with an increased risk of *Leptospira* infection, and that infections in this area can occur year-round (35). Infection is also often associated with occupational activity such as working in civil construction, working with garbage removal, and informal employment (9), the latter was also identified in our study. We also identified individual exposures related to the home environment such as contact with mud, standing water in the vicinity of the home, and especially sewage cleaning, which was associated with an increased risk of infection in both analysis and has been reported as a risk factor for *Leptospira* infection (9, 19).

This study has some limitations that should be considered. The sample size of the quarterly analysis was not ideal for some of our analysis. Longitudinal cohort analysis are logistically and financially troublesome, which is reflected on the few studies conducting such experiments on leptospirosis and the choice to make biannual or annual measurements (9, 18, 19). For that reason, we decided to select a sample of participants who had confirmed infection at the time the biannual survey was carried out. In addition, 35% of subjects in the subcohort did

not complete quarterly follow-up, primarily due to moving out of the study area, which is a common issue in longitudinal studies. Despite those limitations, we were able to identify significant differences in our analysis that agreed with previous studies, indicating the validity of our results. The MAT is the gold standard test recognized by the WHO, but it is a laborious test, subjective and requires experience from the reader. Further, the MAT does not differentiate past from current infection. Those limitations from the MAT are a common feature for several serological assays and always present on leptospirosis studies (30, 36, 37). To minimize impacts on MAT results in our study, only a well-trained and experienced technician was responsible for all readings. Our group has been working in Salvador, Brazil and in the community of Pau da Lima, where this study was conducted, for over 20 years. Since then, we have reduced our panel of MAT strains given the extensive knowledge of circulating strains and reservoirs (9, 18, 19). Our previous studies have shown that over 90% of severe cases of leptospirosis (15) and 90–98% of infections in the community are related to *L. interrogans* serovar Copenhageni. Furthermore, 80% of rats captured in the community (9, 18, 19, 38) were culture positive for leptospirosis, and the serovar Copenhageni was the only one isolated (39). The focus of our study was to understand the role of agglutinating antibodies on the naturally acquired immunity against reinfection, and to have statistical power our analysis were based on the most prevalent serovar in our study site, *L. interrogans* serovar Copenhageni. For those reasons we didn't evaluate agglutinating antibodies for other serovars, including *L. biflexa* serovar Patoc, commonly used as a control. Titers of 1:25 or 1:50, as well as higher titers, were directed against this serovar in our precious studies (9, 18, 19), indicating that this cutoff was a specific and more sensitive criteria for identifying prior infections in a region where a single serovar agent is circulating. Our study site has geographical and social-demographic features that are very similar to other regions of the world where leptospirosis is a problem. Furthermore, the *L. interrogans* is the most common species related to human cases of leptospirosis around the globe (14). Although our results can be generalized to the context of urban leptospirosis worldwide, considerations should be made given recent reports in a mice model that different strains can lead to different levels of immune responses (14).

In summary, we reported antibody kinetics in individuals from an endemic area for leptospirosis showing that frequent exposure to the contaminated environment is an important factor on the infection and reinfection rates of the disease, which are directly affected by the time of intersample collection. Our study also suggested a rapid decay of the humoral response related to agglutinating antibodies and a short-lived naturally acquired immunity against reinfections. Furthermore, our results indicated that serological surveys may be underestimating the burden of *Leptospira* infection and potentially the risk for disease. Further studies are needed to evaluate memory B cells and to assess the humoral response of individuals with previous leptospirosis infections, that could help better to understand the naturally acquired immunity of this important neglected disease and close the knowledge gap on correlates of immunity that can be used to improve prevention.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Yale University Institutional Review Board Research Ethics Committee of the Instituto Gonçalo Moniz Certificate of Presentation for Ethical Appreciation (CAAE). Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JC: conception, methodology, and writing of the original draft. NN: data curation, data analysis, and review. GS, RV, AM, and JS: investigation and review. FC, AK, and MR: funding acquisition, investigation, methodology, and review. EW: funding acquisition, investigation, methodology, proofreading, supervision, writing-proofreading, and editing in English. All authors contributed to the revision and editing of the manuscript, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.862378/full#supplementary-material

Supplementary Figure S1 | Titration curves in log 10 of individuals classified in the quarterly analysis (•) as infection (individual 14 to individual 21) and reinfection

(Individuals 22 and 23) at different times (days) of collection, compared to the bi-annual analysis (,).

Supplementary Table S1 | Anti-leptospira antibody titers distributed by classification and collection period.

Supplementary Table S2 | Characteristic of the subcohort group comparing the stratified analysis of infection based on the quarterly analysis follow-up.

Supplementary Table S3 | Characteristic of the subcohort group comparing the stratified analysis of infection based on the biannual analysis follow-up.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5 **DISCUSSÃO**

A leptospirose é uma doença endêmica em várias regiões do mundo e apresenta uma grande variedade de manifestações clínicas (WHO, 2003). Embora o mecanismo de proteção na leptospirose ainda não seja totalmente conhecido, estudos demonstraram a contribuição das respostas imune humoral e celular contra a doença (ADLER; FAINE, 1978; FAINE et al., 1999). Entretanto, pouco se sabe se indivíduos de áreas endêmicas desenvolvem imunidade protetora adquirida naturalmente contra infecções ou doenças graves, já que a exposição ao ambiente contaminado com *Leptospira* é frequente (TUERO; VINETZ; KLIMPEL, 2010). Compreender a resposta imune humoral envolvida na doença é um passo importante para obter informações que possam auxiliar no desenvolvimento de vacinas mais eficazes e duradouras contra a leptospirose.

No primeiro manuscrito desta tese são apresentados resultados de uma investigação que avaliou o padrão sazonal na ocorrência de infecções por *Leptospira* em Salvador-BA. Tradicionalmente, a leptospirose está associada a chuvas e inundações no mundo e no Brasil (COSTA et al., 2015; KO et al., 1999; LAU et al., 2010). Nossos resultados mostraram que a leptospirose está associada à precipitações que ocorrem em ambientes urbanos e que a exposição ao ambiente contaminado com *Leptospira* pode ocorrer durante todo o ano. Estudos anteriores relatam que chuvas e inundações podem ser importantes fatores de risco para a leptospirose humana pois a chuva frequente pode liberar solo contaminado por *Leptospira* na água, propiciando um ambiente ideal para a sobrevivência da bactéria (DHEWANTARA et al., 2019; LAI et al., 2017; LAU et al., 2012; MWACHUI et al., 2015). Epidemias de leptospirose urbana estão associadas a chuvas intensas, afetando principalmente comunidades vulneráveis. Devido a precárias condições sanitárias e de infraestrutura e grande infestação de roedores, essas comunidades fornecem um cenário ideal para a transmissão da leptospirose (FELZEMBURGH et al., 2014; HAGAN et al., 2016; KO et al., 1999; REIS et al., 2008). Nossos resultados mostraram que a incidência cumulativa na infecção subclínica foi mais elevada, contrastando com o padrão clássico de leptospirose grave relatado pela literatura (BHARTI et al., 2003; KO et al., 1999; LAU et al., 2012), sugerindo que o impacto da leptospirose é subestimado. Este estudo apresentou algumas limitações, como o uso de apenas um critério para definição de infecção o que impossibilitou determinarmos o momento preciso da infecção, além de realizar o inquérito em uma única comunidade da cidade de Salvador.

No segundo manuscrito da tese, é apresentada a taxa de decaimento de títulos de anticorpos anti-*Leptospira* medidos por intervalos de coletas entre seis e doze meses. Nossos resultados mostraram um número maior da taxa média de infecção semestral de leptospirose quando comparada a análises convencionais. Entretanto, esta diferença foi ainda maior quando comparado com a análise anual. Estudos anteriores identificaram que moradores dessa comunidade apresentavam fatores de risco para infecção como a constante exposição ao ambiente contaminado com leptospiras e alta mobilidade de seus habitantes (FELZEMBURGH et al., 2014; HAGAN et al., 2016; OWERS et al., 2018; REIS et al., 2008) e isso pode afetar o cálculo preciso das taxas de decaimento levando a possibilidade de determinar corretamente a taxa de incidência. Esse artigo apresentou limitações como uso de um único conjunto de dados publicados sobre estudos longitudinais em humanos utilizando o MAT para ajuste do modelo.

No terceiro manuscrito desta tese, são apresentadas a cinética de anticorpos e fatores associados a exposição a leptospirose em indivíduos da comunidade de Pau da Lima em Salvador, mediante comparação entre análise sorológica semestral e trimestral. Em nosso estudo, utilizamos como marcador de infecção a presença de anticorpos aglutinantes anti-*Leptospira* detectados no MAT, teste considerado padrão-ouro pela OMS para o diagnóstico da leptospirose. Entre os 72 indivíduos recrutados para a subcoorte, 65% (47) apresentavam anticorpos anti-*Leptospira*, os quais formaram o grupo de positivos. Os demais 35% (25) foram negativos em todos os períodos examinados, constituindo o grupo controle. Vale destacar que nossos achados demonstraram um maior número de infecções e reinfecções quando utilizada a análise trimestral. Estes achados demonstram a relevância da realização de análises trimestrais para identificação de casos de reinfecção na leptospirose. Uma possível explicação para esta diferença seria que indivíduos avaliados podem estar expostos as mesmas fontes de contaminação ou a comportamentos de riscos que levam a se infectarem. Encontramos também um número variável de infecções e reinfecções nestes indivíduos, sugerindo que a resposta humoral em nossa população é curta e não protege contra a reinfecção. Apesar de estudos relatarem que a imunização com leptospiras vivas atenuadas e mortas protegem contra a infecção no modelo animal (ADLER; DE LA PEÑA MOCTEZUMA, 2010; MCBRIDE et al., 2005) e que anticorpos contra proteínas estão correlacionados a proteção (WUNDER et al., 2021), poucos estudos relatam sobre a reinfecção naturalmente adquirida. Em um estudo realizado na mesma comunidade foi relatado que exposição prévia e infecção não conferiram uma proteção completa contra uma infecção subsequente (FELZEMBURGH et al.,

2014). Também foi relatado que a proteção contra reinfecção pelo mesmo sorovar *Leptospira* ou por sorovares relacionados é curta em indivíduos com leptospirose (GRILLOVÁ et al., 2020; GUERREIRO et al., 2001; LUPIDI et al., 1991). No presente estudo, identificamos também que independente do tempo de coleta e análise realizada, os fatores de riscos para aquisição de anticorpos anti-leptospiras na nossa população não se modificam e estão associados a exposições demográficas, individuais ou ocupacionais. As principais limitações deste estudo foram o tamanho amostral para a subcoorte e a perda de acompanhamento, o que nos impossibilitou de realizar algumas análises e, a utilização apenas do MAT que é um teste subjetivo. Porém, as diferenças encontradas estão de acordo com estudos anteriores (FELZEMBURGH et al., 2014; HAGAN et al., 2016).

6 **CONCLUSÕES**

- Nossos resultados mostraram a associação entre chuvas e casos de leptospirose;
- A exposição frequente dos indivíduos ao ambiente contaminado com *Leptospira* é um fator importante para as taxas de infecção e reinfecção;
- O tempo de coleta é importante para identificação de casos de infecções e reinfecções;
- A taxa de decaimento de títulos de anticorpos é um critério importante a ser considerado nas taxas de infecção;
- Os dados também sugerem que a resposta humoral com anticorpos aglutinantes contra *Leptospira* é relativamente curta e protege parcialmente contra reinfecção.

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Apêndice A - Artigos

Durante o período de meu doutorado participei ativamente na colaboração de dois trabalhos com estudos em dengue, quatro com estudos em Zika e três com estudos em leptospirose, nos quais sou co-autora. Seguem abaixo:

One day after admission, the patient was discharged with empirically prescribed ciprofloxacin and metronidazole for 7 days. After 4 days, aerobic blood culture was positive for motile, fusiform, gram-negative bacilli, suggestive of strictly aerobic bacteria that could not be identified directly (Figure, panels B, C). After 7 days of incubation under a microaerophilic atmosphere only, a blood subculture isolate was obtained; 23S and 16S rDNA sequencing (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/2/15-0287-Techapp1.pdf) identified this isolate as *H. trogontum*. Of note, use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany) did not enable identification of the bacterium.

No common pathogens were detected in fecal samples. Upper and lower gastrointestinal endoscopic examinations conducted 1 month after discharge revealed no notable abnormalities. No immunocompromised condition was found. At most recent follow-up examination, the patient was free of symptoms.

The genus *Helicobacter* currently comprises 48 formally named species belonging to the gastric or enterohepatic group according to their ecologic niche. *H. trogontum* (enterohepatic group) has been isolated from apparently health animals (rat and piglet intestinal mucosa and swine feces), but its characteristics are typical of pathogenic bacteria ([*2*,*3*], online Technical Appendix). The apparent in vitro susceptibility of the isolate to metronidazole and the favorable patient outcome reported here are in agreement with the finding that metronidazole is an effective treatment for *H. trogontum* infection in rats ([*4*]; online Technical Appendix), but there are no antimicrobial drug susceptibility data for *H. trogontum* isolated from animals. We assume that the immunocompetent patient reported here had chronic colitis caused by *H. trogontum*, followed by an episode of acute colitis with bacteremia after several years of intermittent symptoms.

The rarity of reported *H. trogontum* infections might be linked to the difficulty associated with culturing and identifying the bacterium or to a low level of exposure to this pathogen. The mode of transmission, probably from animals to humans, remains unclear. Methods for isolation and rapid identification of *H. trogontum*, including the updating of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry databases, are needed for further elucidation of its pathogenic properties and the mode of contamination.

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Accuracy of Dengue Reporting by National Surveillance System, Brazil

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To the Editor: Dengue is an underreported disease globally. In 2010, the World Health Organization recorded 2.2 million dengue cases (*1*), but models projected that the number of symptomatic dengue cases might have been as high as 96 million (*2*). Brazil reports more cases of dengue than any other country (*1*); however, the degree of dengue underreporting in Brazil is unknown. We conducted a study to evaluate dengue underreporting by Brazil's Notifiable Diseases Information System (Sistema de Informação de Agravos de Notificação [SINAN]).

From January 1, 2009, through December 31, 2011, we performed enhanced surveillance for acute febrile illness

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RESEARCH ARTICLE

Accuracy of the SD BIOLINE Dengue Duo for rapid point-of-care diagnosis of dengue

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Abstract

Background

Rapid diagnosis tests (RDTs) are easy to carry out, provide fast results, and could potentially guide medical treatment decisions. We investigated the performance of a commercially available RDT, which simultaneously detects the non-structural 1 (NS1) dengue virus (DENV) antigen, and IgM and IgG DENV antibodies, using representative serum samples from individuals in a dengue endemic area in Salvador, Brazil.

Methodology/Principal findings

We evaluated the accuracy of the SD BIOLINE Dengue Duo RDT (Abbott, Santa Clara, USA; former Alere Inc, Waltham, USA) in a random collection of sera. Samples included acute-phase sera from 246 laboratory-confirmed dengue cases and 108 non-dengue febrile patients enrolled in a surveillance study for dengue detection, 73 healthy controls living in the same surveillance community, and 73 blood donors. RDT accuracy was blindly assessed based on the combined results for the NS1 and the IgM test components. The RDT sensitivity was 46.8% (38.6% for the NS1 component and 13.8% for the IgM component). Sensitivity was greater for samples obtained from patients with secondary DENV infections (49.8%) compared to primary infections (31.1%) (P: 0.02) and was also influenced by the result in the confirmatory dengue diagnostic test, ranging from 39.7% for samples of cases confirmed by IgM-ELISA seroconversion between paired samples to 90.4% for samples of cases confirmed by a positive NS1-ELISA. The RDT specificity was 94.4% for non-dengue febrile patients, 87.7% for the community healthy controls, and 95.9% for the blood donors.

Does immunity after Zika virus infection cross-protect against dengue?

Zika and dengue viruses are closely related flaviviruses, with immunological interactions and identical urban, mosquito-borne transmission.[1] Therefore, the recent introduction of Zika virus into the Americas and large-scale exposure of a uniformly previously unexposed population could affect subsequent transmission of dengue virus. This hypothesis had been untested, largely because sufficient epidemiological data were not available from affected locations. We explored this hypothesis in Salvador, the fourth largest city in Brazil (population 2·9 million), where extensive transmission of dengue viruses 1–4[2,3] occurred before the introduction and spread of Zika virus in 2015.[4]

We have done continuous enhanced surveillance of dengue among patients with acute febrile illness in a slum community of Salvador (population 76 352) since January 2009,[2] except for the periods September, 2013, to September, 2014, and August to September, 2016. The surveillance was first interrupted in 2013 with the termination of the supporting research grant; surveillance was restarted in 2014 with funding from a new award. The second interruption was due to the closing of the health centre for maintenance.

Before 2015, the frequency of RT-PCR-positivity for dengue virus followed a pattern of annual second-quarter or third-quarter peaks (figure A). By contrast, a much smaller peak occurred in 2015 during the Zika virus epidemic, and no peak occurred in 2016 and 2017, when RT-PCR positivity for dengue virus was around zero. These findings represented a significant decrease in the frequency of confirmed dengue virus among outpatients with acute febrile illness, from 484 (25%) cases of 1937 before the Zika virus outbreak from January, 2009, to March, 2015, to 43 (3%) of 1334 after the outbreak from April, 2015, to May, 2017 ($p<0·0001$; appendix).

In September, 2014, we augmented our dengue surveillance by adding routine testing for Zika virus and chikungunya virus. Of 1407 patients with acute febrile illness tested for Zika virus, 14 (1%) cases were confirmed by RT-PCR. The first was confirmed in May, 2015, with a peak in the number of cases during April to June, 2015 (11 [4%] of 285 tested patients). By contrast with the minimal detection of dengue in 2015, a large outbreak of chikungunya virus occurred at the surveillance site in the same year. The frequency of RT-PCR-positive or IgM-ELISA-positive cases of chikungunya virus among

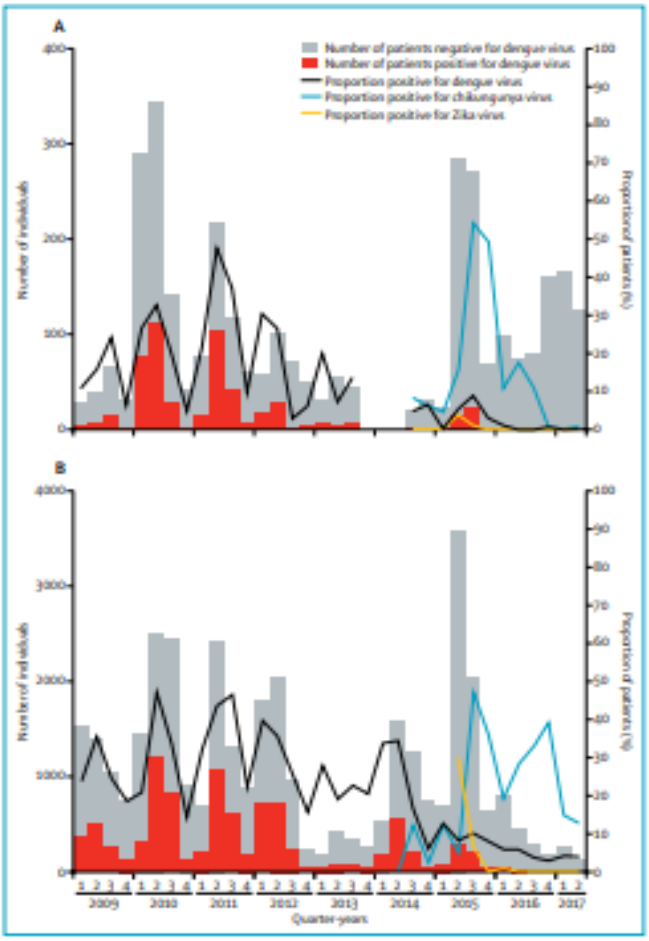

Figure: Quarterly frequency of laboratory-confirmed dengue in Salvador, Brazil, before the appearance of Zika virus (January 2009 to March 2015) and after its appearance (April 2015 to May 2017)
Data are (A) cases of dengue virus infection among patients with acute febrile illness enrolled during enhanced surveillance, confirmed by RT-PCR; and (B) cases of dengue virus infection confirmed by RT-PCR, IgM, or NS1 ELISA, or viral isolation, among patients with suspected dengue from Salvador. Figures also show the frequency of cases of chikungunya virus infection laboratory confirmed by RT-PCR or IgM ELISA, and of Zika virus infection confirmed by RT-PCR.

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See Online for appendix

JEM Journal of Experimental Medicine

ARTICLE

Risk of Zika microcephaly correlates with features of maternal antibodies

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Zika virus (ZIKV) infection during pregnancy causes congenital abnormalities, including microcephaly. However, rates vary widely, and the contributing risk factors remain unclear. We examined the serum antibody response to ZIKV and other flaviviruses in Brazilian women giving birth during the 2015–2016 outbreak. Infected pregnancies with intermediate or higher ZIKV antibody enhancement titers were at increased risk to give birth to microcephalic infants compared with those with lower titers ($P < 0.0001$). Similarly, analysis of ZIKV-infected pregnant macaques revealed that fetal brain damage was more frequent in mothers with higher enhancement titers. Thus, features of the maternal antibodies are associated with and may contribute to the genesis of ZIKV-associated microcephaly.

Introduction

For decades, infection by Zika virus (ZIKV) went either unrecognized or occurred only sporadically and was associated with mild symptoms. ZIKV was detected in Brazil in 2015 and spread rapidly, reaching infection rates exceeding 60% (Zanluca et al., 2015; Netto et al., 2017; Rodriguez-Barraquer et al., 2019). During the Brazilian ZIKV outbreak, it was recognized that congenital infection can cause fetal abnormalities, including visual and hearing impairment, skeletal deformities, and microcephaly, with an overall prevalence of microcephaly estimated at 2.7 to 5.8% of live births from ZIKV-infected pregnancies and with a global rate of adverse outcomes exceeding 40% in some regions (Brasil et al., 2016; Kleber de Oliveira et al., 2016; Rasmussen et al., 2016; Coelho and Krevella, 2017; Hoen et al., 2018). Why some ZIKV-infected pregnant women deliver apparently healthy newborns while others have babies with microcephaly is unknown.

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Impact of preexisting dengue immunity on Zika virus emergence in a dengue endemic region

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Competing interests: EJMN, ETAM., and AIK are authors on a patent application, entitled "Methods and Composition for the Detection of Flavivirus Infections" related to this work (U.S. Provisional Patent Application Serial No. 62/503.201 and US Provisional Patent Application Serial No. 62/608.927). AIK received honoraria from Sanofi-Pasteur for his participation in a conference on Zika vaccines. The authors declare that they have no other competing interests.

Data and materials availability: All relevant data are within the paper and its Supporting Information files. Code to reproduce study findings is available at https://doi.org/10.5281/zenodo.2528486.

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Social determinants associated with Zika virus infection in pregnant women

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Abstract

This study aims to describe the sociodemographic determinants associated with exposure to Zika Virus (ZIKV) in pregnant women during the 2015–2016 epidemic in Salvador, Brazil.

Methods

We recruited women who gave birth between October 2015 and January 2016 to a cross-sectional study at a referral maternity hospital in Salvador, Brazil. We collected information on their demographic, socioeconomic, and clinical characteristics, and evaluated their ZIKV exposure using a plaque reduction neutralization test. Logistic regression was then used to assess the relationship between these social determinants and ZIKV exposure status.

Results

We included 469 pregnant women, of whom 61% had a positive ZIKV result. Multivariate analysis found that lower education (adjusted Prevalence Rate [aPR] 1.21; 95%CI 1.04–1.35) and food insecurity (aPR 1.17; 95%CI 1.01–1.30) were positively associated with ZIKV exposure. Additionally, age was negatively associated with the infection risk (aPR 0.99; 95%CI 0.97–0.998).

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Distinct antibody responses of patients with mild and severe leptospirosis determined by whole proteome microarray analysis

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Abstract

Background

Leptospirosis is an important zoonotic disease worldwide. Humans usually present a mild non-specific febrile illness, but a proportion of them develop more severe outcomes, such as multi-organ failure, lung hemorrhage and death. Such complications are thought to depend on several factors, including the host immunity. Protective immunity is associated with humoral immune response, but little is known about the immune response mounted during naturally-acquired *Leptospira* infection.

Methods and principal findings

Here, we used protein microarray chip to profile the antibody responses of patients with severe and mild leptospirosis against the complete *Leptospira interrogans* serovar Copenhageni predicted ORFeome. We discovered a limited number of immunodominant antigens, with 36 antigens specific to patients, of which 11 were potential serodiagnostic antigens, identified at acute phase, and 33 were potential subunit vaccine targets, detected after recovery. Moreover, we found distinct antibody profiles in patients with different clinical outcomes: in the severe group, overall IgM responses do not change and IgG responses increase over time, while both IgM and IgG responses remain stable in the mild patient group. Analyses of individual patients' responses showed that >74% of patients in the severe group had significant IgG increases over time compared to 29% of patients in the mild group. Additionally, 90% of IgM responses did not change over time in the mild group, compared to ~51% in the severe group.

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Prospective evaluation of accuracy and clinical utility of the Dual Path Platform (DPP) assay for the point-of-care diagnosis of leptospirosis in hospitalized patients

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Abstract

Early detection of leptospirosis with field-ready diagnostics may improve clinical management and mitigate outbreaks. We previously validated the point-of-care Dual Path Platform (DPP) for leptospirosis with sera in the laboratory. This prospective study compares the diagnostic accuracy and clinical utility of the DPP using finger stick blood (FSB) against the serum DPP, venous whole blood (VWB) DPP, IgM-ELISA, and clinical impression. We sequentially enrolled 98 patients hospitalized for acute febrile illnesses, of which we confirmed 32 by leptospirosis reference tests. Among syndromes consistent with classic leptospirosis, the FSB DPP showed similar sensitivity and specificity (Se 93% and Sp 80%), and positive and negative predictive values (PPV 74% and NPV 95%), to VWB DPP (Se 96%, Sp 75%, PPV 68%, and NPV 97%), serum DPP (Se 85%, Sp 87%, PPV 79%, and NPV 91%) and IgM-ELISA (Se 81%, Sp 100%, PPV 100%, and NPV 90%). The FSB DPP provided a favorable likelihood ratio profile (positive LR 4.73, negative LR 0.09) in comparison to other assays and clinical impression alone. Additionally, we identified four of five leptospirosis-associated meningitis patients by whole blood DPP, none of which clinicians suspected. This demonstrates potential for the DPP in routine detection of this less common syndrome. The FSB DPP demonstrated similar discrimination for severe human leptospirosis compared with serum assays, and it is a simpler option for diagnosing leptospirosis. Its performance in other epidemiological settings and geographic regions, and for detecting atypical presentations, demands further evaluation.

PLOS NEGLECTED TROPICAL DISEASES

SYMPOSIUM

Severe leptospirosis after rat bite: A case report

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Presentation of case

A 43-year-old woman presented to an emergency department in Salvador, Brazil, with a two-day history of fever (39.5˚ to 40.0˚C), chills, headache, arthralgia, myalgia, and loss of appetite in the setting of a recent rat bite. She had no previous relevant medical history but reported a street-rat bite on her right ankle 13 days prior to presentation (Fig 1). The rat bite occurred while she was walking to a drugstore in the early evening in December 2014 in a medium-income neighborhood of Salvador, a coastal city in the northeast of Brazil. Shortly after the incident, she went to an urgent care unit where she received tetanus and rabies vaccines and wound care. She denied exposure to other potentially leptospires-contaminated environments, such as water or mud. When the symptoms began, she was seen at the hospital where she received medical examination and laboratory evaluation. Her complete blood count (Day 1) showed discrete anemia, leukocytosis with neutrophilia, and thrombocytopenia (Table 1). Her urinalysis showed hematuria. The erythrocyte sedimentation rate was 24 mm^3/hr, creatine phosphokinase was 1,182 U/L, and no other pertinent findings were reported. Blood culture showed no growth, and a rapid dengue test was nonreactive. She received intravenous fluids, muscle relaxants, and analgesics and was discharged without a clear diagnosis. Persistent symptoms brought the patient back to the hospital the next day (Day 2) complaining of shortness of breath, diffused myalgia, arthralgia, odynophagia, dry mouth, and hemoptysis as well as cutaneous rashes. Clinical examination recorded a temperature of 38.0˚C, blood pressure of 117/72 mmHg, heart rate of 100 beats per minute, respiratory rate of 16 breaths per minute, and oxygen saturation of 99% breathing room air, in addition to dehydration. She was admitted to the hospital that same day—antibiotics (ceftriaxone) and supportive measures were initiated. On Day-3, she developed shortness of breath and crepitus on thorax auscultation at the base of her right lung and had 82% of oxygen saturation at room air. The patient was admitted to the intensive care unit (ICU) for noninvasive respiratory support. Thorax computed tomography and X-ray revealed bilateral diffused consolidation with air bronchogram and a posterior basal laminar stroke of her left lung (Fig 2). The antibiotics were changed to moxifloxacin and cefepime. Oseltamivir and corticosteroids were introduced. Additionally, during Day 3 in the ICU, the patient presented with hemoptysis and pulmonary congestion, likely associated to hypervolemia, which were resolved by the Day 4. The patient was discharged on Day 6 with complete resolution of fever and respiratory symptoms. A definitive diagnosis of leptospirosis was made based on the epidemiological history of rat bite, compatible clinical symptoms, and laboratory tests. A positive immunoglobulin M (IgM) ELISA (Bio-Manguinhos, Rio de