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ECO-EPIDEMIOLOGÍA E VULNERABILIDADE DA FEBRE MACULOSA NO ESTADO DO RIO DE JANEIRO

DIEGO CAMILO MONTENEGRO LÓPEZ

Rio de Janeiro
Agosto 23 de 2017
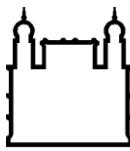
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DIEGO CAMILO MONTENEGRO LÓPEZ

Eco-epidemiología e vulnerabilidade da febre Maculosa no estado do Rio de Janeiro

Tese apresentada ao Instituto Oswaldo Cruz como parte dos requisitos para obtenção do título de Doutor em Medicina Tropical

Orientador (es): Prof. Dr. Reginaldo Peçanha Brazil.
Prof. Dr. Gilberto Salles Gazeta

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ii

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Rio de Janeiro, 23 de agosto de 2017

i

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Declaramos, para fins curriculares, que Diego Camilo Montenegro Lopéz, sob orientação do Dr. Reginaldo Peçanha Brazil e Dr. Gilberto Salles Gazeta, foi aprovado em 23/08/2017, em sua defesa de tese de doutorado intitulada: **"Eco-epidemiologia e vulnerabilidade da febre maculosa no estado do Rio de Janeiro"**, área de concentração: Diagnóstico, Epidemiologia e Controle. A banca examinadora foi constituída pelos Professores: Drª Maria Halina Ogrzewalska - IOC/FIOCRUZ (presidente), Dr. Adriano Pinter dos Santos - Sucen/SP, Dr. Ary Elias Aboud Dutra UCB/RJUCB/RJ, Drª. Monica de Avelar Figueiredo Mafra Magalhães - ICICT/FIOCRUZ-RJ, Dr. Flávio Luis de Mello - UFRJ-RJ e como suplentes: Drª. Marinete Amorim - IOC/FIOCRUZ e Drª. Heloiza Helena de Oliveira Morelli Amaral - SES/RJ.

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INSTITUTO OSWALDO CRUZ
ECO-EPIDEMIOLOGÍA E VULNERABILIDADE DA FEBRE MACULOSA NO ESTADO DO RIO DE JANEIRO
RESUMO
<u>**TESE DE DOUTORADO EM MEDICINA TROPICAL**</u>

Diego Montenegro López

A Febre Maculosa (FM) é uma doença causada por bactérias e transmitida por vetores, especialmente carrapatos, com um dos maiores impactos no Brasil pela quantidade de mortes que provoca, em relação ao número de pessoas infectadas. É relatada no Estado do Rio de Janeiro (RJ) desde a década de 40, havendo comprovação de óbitos em várias regiões do Estado. Apesar de seu interesse para a saúde pública, pouco se conhece a respeito dos fatores que permitem a instalação ou ampliação dos focos de transmissão epidêmicos e epizoóticos, não se tem uma avaliação do Sistema de Informação de Agravos de Notificação (SINAN) na captação, diagnostico e confirmação de casos suspeitos para FM e também não uma avaliação da vulnerabilidade espacial pela FM no RJ. Situações que serão tratadas no presente trabalho acadêmico. Na primeira abordagem, identificamos artrópodes infectados com *Rickettsia felis*, *R. bellii* e *R. Rickettsii*, sendo modelados por seus hospedeiros específicos. A relação *R. rickettsii*-vector-hospedeiro foi mais evidente no parasitismo específico, sugerindo que a associação entre cães, gado, cavalos, capivaras e seus principais ectoparasitas, *Rhipicephalus sanguineus* e *Ctenocephalides felis*, *R. microplus*, *Dermacentor nitens* e *Amblyomma dubitatum*, respectivamente, têm um papel fundamental na dinâmica da transmissão de *R. rickettsii* em ciclos enzoóticos e na manutenção de populações de vetores infectados, que proporcionam a existência de áreas endêmicas com a oportunidade de virem surtos epidêmicos de FM no RJ. O parasitismo em humanos só foi confirmado por *Amblyomma sculptum* infectado com *R. rickettsii*, o que reforça a importância dessa espécie como vetor do patógeno no Brasil. No segundo e terceiro cenários verificamos que a dinâmica da epidemiologia é muito heterogênea no tempo e no espaço, com surtos em determinados momentos, com altas taxas de mortalidade e tempos de silêncio epidemiológico, alterando seu perfil de doença rural para doença urbana como esta acontecendo em todas as áreas endêmicas do Brasil. Nos últimos 34 anos, houve 990 notificações com 116 casos confirmados de FM residentes no 42,39% dos municípios do estado. Se evidência que próximo do 12% dos casos notificados se confirmam como FM, 3% como dengue, 1,6% como leptospirosis e 0,7% correspondem à alergia à picada do carrapato. Cenários de fluxo de pacientes entre os sítios de infecção, residência e atenção médica entre estados fronteiriços e dentro do RJ também acontece. Confirmamos que não é possível fazer uma classificação diagnóstica dos casos suspeitos de FM através dos sinais e sintomas clínicos, empregando técnicas de redes neurais, situação associada, em parte, pela qualidade da informação que é depositada no SINAN. A vulnerabilidade espacial na infecção humana com Rickettsias patogênicas transmitidas pelos carrapatos pode ser analisada em três níveis: **i.** O individual ou LPI; **ii.** A população ou município; e **iii.** O ecossistema ou estado. Este estudo pode ser adaptado a diferentes cenários eco-epidemiológicos de febre maculosas no Brasil e nas Américas.
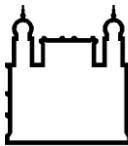
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ECO-EPIDEMIOLOGY AND VULNERABILITY TO SPOTTED FEVER IN THE STATE OF RIO DE JANEIRO

ABSTRACT
PHD THESIS IN MEDICINA TROPICAL)

Diego Montenegro López

Spotted fever (SF) is caused by a bacterium that is transmitted by vectors, especially ticks. It has a significant impact in Brazil due to the number of deaths it causes relative to the number of people who become infected. It has been reported in the state of Rio de Janeiro (RJ) since the 1940s, with evidence of deaths due to SF in several regions of the State. Despite its public health significance, little is known about the factors that allow the establishment or expansion of epidemic and epizootic outbreaks. Furthermore, there is no long-term epidemiological evaluation of the disease by the Epidemiological Surveillance System (SINAN), incorporating information regarding capture, diagnosis and confirmation of suspected cases, and no assessment of spatial vulnerability to SF in RJ; situations that are addressed in the present work. In our first experiment, we infected arthropods with *Rickettsia felis*, *R. bellii* and *R. rickettsii*, and modeled their host specificity. The *R. rickettsii*-vector-host relationship was most evident in specific parasitism, suggesting that associations between dogs, cattle, horses and capybaras, and their main ectoparasites, *Rhipicephalus sanguineus* and *Ctenocephalides felis, R. microplus, Dermacentor nitens,* and *Amblyomma dubitatum*, respectively, have a key role in the dynamics of *R. rickettsii* transmission in enzootic cycles and the maintenance of infected vectors, which facilitates the existence of endemic areas with the potential of epidemic outbreaks of SF in RJ. Parasitism of humans was only confirmed for *Amblyomma sculptum* infected with *R. rickettsii*, which reinforces the importance of this species as a vector of the pathogen in Brazil. In our second and third experiments, we verified that the epidemiological dynamics of SF are very heterogeneous in time and space, with moments of outbreaks with high rates of mortality, yet other times that are epidemiologically silent, and a changing profile from a rural to an urban disease, as are all of the endemic areas of Brazil. Over the last 34 years there have been 990 notifications of SF, with 116 confirmed cases of residents in 42.39% of the municipalities of RJ. Approximately 12% of the notified cases were confirmed as SF, 3% as dengue, 1.6% as leptospirosis and 0.7% as tick bite allergy. Patient flow among sites of infection, residency, and medical care, within RJ and among bordering states, also occurs. We confirmed that it is not possible to diagnose suspected cases of SF through clinical signs and symptoms using neural network techniques, a situation associated in part with the quality of information that is deposited in SINAN. Spatial vulnerability of human infection with tick-borne pathogenic *Rickettsia* can be analyzed at three levels: (i) the individual or probable areas of infection; (ii) the population or municipality; and (iii) the ecosystem or state. This study can be adapted to different eco-epidemiological scenarios of SF in Brazil as well as other countries in the Americas.

ÍNDICE

1 INTRODUÇÃO

Histórico das Rickettsioses e a Febre Maculosa (FM)

Rickettsioses é um grupo de doenças infecciosas causadas por bactérias patogênicas que fazem parte da família Rickettsiaceae, Ordem Rickettsiales. Entretanto, seguindo a nomenclatura internacional (1), no presente trabalho será utilizado o termo rickettsioses apenas pelas doenças produzidas pelo gênero *Rickettsia*.

As rickettsioses estão presentes em quase todos os continentes, em focos naturais ou áreas com casos permanentes (endêmicos), podendo emergir com impactos negativos na saúde humana (epidêmica) com alta taxa de letalidade. Estão entre as doenças compartilhadas entre animais e humanos (zoonoses) e vêm despertando grande interesse científico na área das ciências biomédicas em função de sua reemergência em várias regiões do mundo, sendo, assim, definidas como um problema de Saúde Pública (2,3).

A heterogeneidade de agentes etiológicos da Ordem Rickettsiales produz variadas doenças em humanos que, em termos didáticos, podem ser agrupadas em:

1. Febres Tíficas: Tifo Epidêmico, doença de Brill-Zinsser, Tifo Endêmico ou Murino, Tifo das Malezas e Febre Quintana.

2. Febres Exantemáticas ou Manchadas: Existe um grande número de febres exantemáticas no mundo, entre as mais conhecidas estão a FM, a Febre Botonosa Mediterrânea e Tibola.

A FM ganha relevância, por ser endêmica nas Américas, com 3 focos clássicos: 1) A Febre Maculosa das Montanhas Rochosas (4), nos Estados Unidos da América; 2) A Febre Maculosa Brasileira (5); 3) e a Febre de Tobia, de ocorrência na Colômbia (6).

A primeira descrição clínica da FM foi feita em 1899 por Maxcy em um caso ocorrido na região montanhosa do noroeste norte-americano (7). Mas apenas em 1906 começou-se a associar bactérias ao ciclo de transmissão da doença (4) nos Estados Unidos. A partir da década de 30 a doença passou a ser identificada focalmente em diversos países da América do Sul.

No Brasil a doença foi reconhecida pela primeira vez no Estado de São Paulo por Piza em 1929 (8). A partir daí foram diagnosticados casos no RJ e Estado de Minas Gerais (9). Porém, somente no ano de 2001 foi considerada, pelo Ministério da Saúde, uma doença de notificação compulsória (10). A partir desse ano até 2015 se têm notificação de casos de FM na maioria das unidades federativas do país, com casos confirmados em, aproximadamente, 44% (12/27) do território brasileiro (11).

Elementos da Cadeia de Transmissão das *Rickettsia* Causadoras da Febre Maculosa

1.2.1. *Agentes Etiológicos*

As *Rickettsia* estão caracterizadas como proteobactérias gram-negativas e intracelulares obrigatórias que infectam células endoteliais de animais e o homem

(**Figura 1**), com complicações sistêmicas que podem ser fatais se não houver tratamento adequado e oportuno (12,13).

Na atualidade são reconhecidas 31 espécies de *Rickettsia* (http://www.bacterio.net/rickettsia.html)*,* ao menos 18 delas associadas a casos humanos de doença (14,15).

O permanente desenvolvimento do conhecimento, especialmente na área da biologia molecular, tem influenciado significativamente as constantes revisões taxonômicas e filogenéticas, com diferentes proposições para *Rickettsia* (17–19).

Classicamente, as espécies do gênero *Rickettsia* estão subdivididas entre os Grupos Tifo (GT), Grupo Ancestral (GA) e Grupo Febre Maculosa (GFM). O GT é composto por *Rickettsia prowazekii* transmitida por piolho (produz Tifo Epidêmico) e *Rickettsia typhi* veiculada por pulgas (ocasiona Tifo Murino ou Tifo Endêmico), de ampla distribuição mundial. O GA inclui *Rickettsia canadensis* e *Rickettsia belli*, com patogenicidade desconhecida (20,21).

O GFM é o de maior relevância epidemiológica nas Américas (13,22), sem, contudo, ser considerado prioridade na Saúde Pública na maioria destes países.
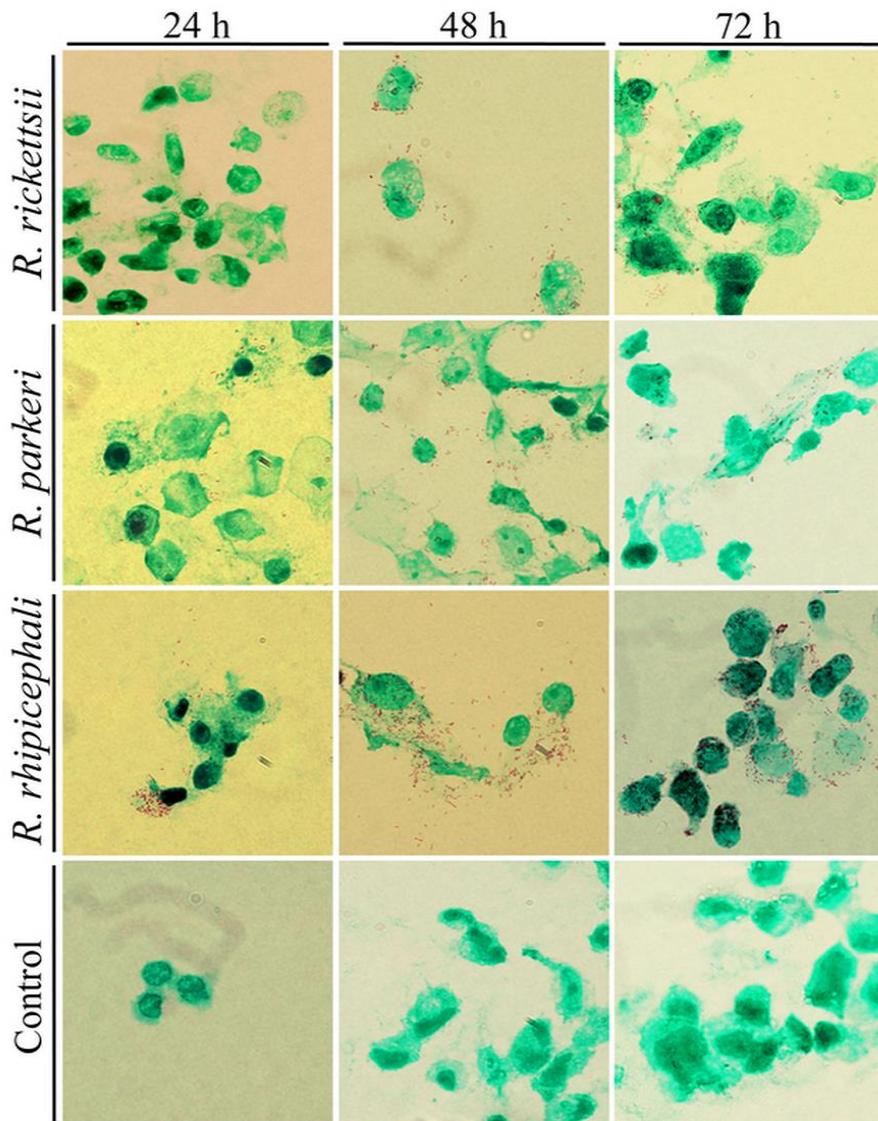
Figura 1: Fotomicrografias que ilustram a presença de *Rickettsia* spp. do Grupo Febre Maculosa (pontos vermelhos) em células Vero e células Vero não infectadas (Controle), tingido de acordo com o método de Giménez (Giménez 1964) (1000 ×Ampliação, microscópio óptico Olympus DP72) a 24, 48 e 72 h pós inoculação bacteriana. Créditos: Arannadia Silva (23).

Durante quase todo o século XX, a *Rickettsia rickettsii* foi considerada a única associada à doença humana nas Américas (22). Atualmente se conhecem cinco

espécies patogênicas (*Rickettsia africae, Rickettsia felis, Rickettsia massilae, Rickettsia parkeri, Rickettsia philipii* e *R. rickettsii*) e nove espécies circulando em carrapatos, em áreas com casos de rickettsioses; todas com patogenicidade desconhecida (*Rickettsia amblyommatis, Candidatus Rickettsia andeanae, R. belli, R. canadensis, Candidatus Rickettsia cooleyi, Rickettsia montanensis, Rickettsia monteiroi, Rickettsia peacokii* e *Rickettsia rhipicephali*) (14).

No Brasil, entre as doenças produzidas por agentes etiológicos transmitidos por carrapatos, a mais prevalente e de interesse na Saúde Pública, são a Febre Maculosa Brasileira (FMB) produzida pela *Rickettsia rickettsii* e a FM ocasionada por outras espécies de *Rickettsia* consideradas emergentes, por exemplo *Rickettsia* cepa Mata Atlântica (24–26). Porém, no presente trabalho todas serão tratadas como Febre Maculosa (FM). Outras espécies consideradas não patogênicas (*R. amblyommatis, R. rhipicephali, Rickettsia monteiroi, C. R. andeanae*) foram identificadas em áreas com casos humanos confirmados de FM, em diferentes espécies de vetores e hospedeiros (27–33). Isso indica à complexidade do ciclo enzoótico e epidêmico da FM, bem como a diversidade de carrapatos potenciais envolvidos em função da variedade de cenários eco-epidemiológicos.

1.2.2. *Vetores-reservatórios*

Depois dos mosquitos, os carrapatos, todos hematófagos, tem a maior capacidade de transmitir patógenos aos humanos (34). Porém, os carrapatos ganham na heterogeneidade de agentes etiológicos (bactérias, vírus, protozoários e helmintos) que podem ser veiculados durante o repasto sanguíneo sobre um hospedeiro (35,36), principalmente mamífero. Aproximadamente 10% das espécies

de carrapatos conhecidas (900 espécies) tem importância na Saúde Pública e Medicina Veterinária (35).

Os carrapatos são considerados ectoparasitos, porque parasitam o exterior de variados vertebrados, dos quais se alimentam para sobreviver. São organismos pertencentes ao reino Animalia, filo Arthropoda, classe Arachnida, ordem Ixodida, com duas famílias presentes no Brasil: Ixodidae e Argasidae (37).

Os Argasídeos, também conhecidos como carrapatos moles, principalmente pela ausência do escudo dorsal, estão conformados por 195 espécies no mundo todo, com oito espécies representantes da família no Brasil (37) . Geralmente estão parasitando animais silvestres como aves, morcegos e roedores. Os Ixodídeos é o grupo mais rico em espécies (650 no mundo e 47 no Brasil) (37), são denominados carrapatos duros, engloba a maioria dos carrapatos de importância médico-veterinária, especialmente incriminados na transmissão dos patógenos causadores da FM.

Na natureza, a manutenção do ciclo da *R rickettsii* e demais bactérias do GFM é garantida pela capacidade dos carrapatos atuarem como vetores, reservatórios e/ou amplificadores. Os carrapatos, tanto machos como fêmeas, podem adquirir e transmitir os patógenos durante o ato de alimentação sobre um hospedeiro. Também pode ocorrer infecção transovariana da bactéria (fêmea infectada passa para os ovos) e transestadial (estádio-estádio biológicos), a partir da cópula, além da alimentação simultânea de carrapatos infectados com não infectados em animais com suficiente rickettsisemia (24,38,39). Uma vez o carrapato infectado pode permanecer infectado durante tudo seu ciclo de vida.

6

O tempo de vida do carrapato varia entre as espécies e as condições do habitat, porém, em geral, superam um ano de vida. Durante esse tempo pode fazer vários repastos sanguíneos, ovipostura e transmitir as rickettsias para uma grande diversidade de mamíferos (Goodman et al. 2005).

Rickettsia tem sido identificadas numa ampla heterogeneidade de carrapatos (14–16). Porém, poucas espécies de carrapatos tem demostrado funcionar como amplificadores e vetores, especialmente do GFM (14,15,21).

No Brasil, *Amblyomma sculptum*, *Amblyomma aureolatum* e *Amblyomma dubitatum* são as principais espécies de importância epidemiológica, vinculadas à transmissão de *R. rickettsii* (24,37,40).

Recentes trabalhos (11,26,41,42) indicam que a dinâmica da FM nos estados endêmicos do Brasil é de caráter sazonal. Essa sazonalidade tem sido associada à dinâmica populacional de espécies do gênero *Amblyomma,* especialmente a maior densidade de larvas e ninfas de *A. sculptum* (24,43–47). Esse carrapato é considerado o principal vetor de *R. rickettsii* para a população humana no Brasil (24–26,42).

Na atualidade, no Brasil, cinco espécies de carrapatos podem ser catalogadas no estatos de carreadores de *R. rickettsii* em áreas com casos humanos de FM: *A. sculptum* (48,49)*, A. aureolatum* (50)*, A. dubitatum* (51)*, Rhipicephalus sanguineus* (28,29) e *Rhipicephalus microplus* (42)*.* Outras espécies (*Rickettsia* cepa Mata Atlântica) associadas a casos humanos, tem sido

identificadas no carrapato *Amblyomma ovale* (52) enquanto *R. parkeri* em *A. sculptum* (49).

Embora vários intentos em demostrar, experimentalmente, a capacidade vetorial de algumas espécies de carrapatos e de vertebrados funcionar como amplificadores e reservatórios de *R. rickettsii*, somente tem sido comprovado esse fenômeno em *A. sculptum*, associada a capivaras (*Hidrochaeris hidrochaeris*) e *Didelphis aurita* (53,54), assim como tem sido comprovado *A. aureolatum* e *R. sanguineus* associadas a cães (55,56).

No estado do Rio de Janeiro, considerado endêmico para FM, tem-se identificado a circulação de três espécies de *Rickettsia* em diferentes espécies de vetores (transmite a bactéria) e carreadores (não amplifica e não transmite a bactéria): *R. rickettsii* nos carrapatos *A. sculptum, A. dubitatum, R. sanguineus* e na pulga *Ctenocephalides felis*; *Rickettsia belli* em *A. dubitatum* e *Rickettsia felis* em *C. felis* (29,42,51,57).

Embora os carrapatos como fator biológico são os responsáveis diretos pela incidência da FM em populações humanas, a ecologia das populações dos carrapatos, além de ser influenciada por fatores bióticos (hospedeiros, predadores, tipo de vegetação, fragmentação do habitat, perdidas ou migração de animais, etc) também são afetados por fatores abióticos (temperatura, humidade, precipitações, altitude, etc) (38,58–60,57,61–65). Por tanto, esse conjunto de fatores são fundamentais na variação espaço-temporal do risco na transmissão de patógenos aos humanos (66–68), incluindo a FM.

1.2.3. *Hospedeiros e amplificadores*

A origem da hematofagia nos carrapatos ocorreu no Cretáceo tardio e foi estimulada pela radiação biológica das aves es mamíferos placentários, para ocupar novos nichos (69).

Através do processo evolutivo ocorreram adaptações, especialmente do aparelho bucal e substâncias bioquímicas, permitindo a hematofagia em todos os estádios biológicos (larvas, ninfas e adultos) e a capacidade de se alimentar de vertebrados terrestres, principalmente mamíferos (16,69–71).

O processo de alimentação dos carrapatos pode ter impacto negativo para seus hospedeiros, seja pela liberação de toxinas, exanguinação ou transmissão de bioagentes que podem causar enfermidades (16,70,72); o que leva sua importância para a Saúde Pública e Médica Veterinária.

Eventualmente, animais sinantrópicos (associados a humanos) e/ou domésticos, que atuam como hospedeiros primários de carrapatos, podem se infestar com carrapatos infectados com *Rickettsia* em ambientes silvestres, introduzir e manter linhagens de carrapatos infectadas em ambientes antrópicos, podendo estabelecer, assim, um novo foco epidêmico (63,73).

Logo, em um dado ecótopo, a disponibilidade de espécies de hospedeiros vertebrados e vetores, bem como sua suscetibilidade às infecções por *Rickettsia*, podem variar no espaço e ao longo do tempo.

Dessa forma, a dinâmica do ciclo enzoótico das *Rickettsia* do GFM envolve múltiplos grupos de vetores e mamíferos que podem coexistir na mesma área, compartilhando ou não de elementos eco-epidemiológicos. Além disso, os

diferentes tipos de atividades humanas e sua conexão à atividade sazonal dos vetores influenciam a manifestação epidêmica da rickettsiose no foco natural.

Algumas espécies de carrapatos podem parasitar mais de uma espécie de hospedeiro ao longo de seu ciclo de vida (ciclo trioxeno) enquanto outras parasitam um único hospedeiro durante toda sua vida (ciclo monoxeno) (37,42).

Na atualidade existem poucos mamíferos reconhecidos como amplificadores da *R. rickettssi*, como é o caso da capivara e o marsupial *Didelphis aurita* (53,54). A bactéria pode infectar, se multiplicar, circular no sangue do animal (rickettsemia) por tempo suficiente para que novos indivíduos e espécies de carrapatos possam adquirir a bactéria no ato da alimentação sobre ele.

Os cães são sabidamente reconhecidos como animais que podem se infectar com *R. rickettsii,* amplificar essas bactérias e funcionar como reservatórios, adoecer e morrer pela FM (55,56); por isso são considerados como animais sentinelas em áreas com ciclo enzoóticos.

1.3 Patogenia e Manifestações Clínicas em Humanos

Todos os seres humanos estão sujeitos à infecção por rickettsias, e a doença estimula imunidade sólida e duradoura nos que se recuperam, prevenindo reinfecções (74).

As rickettsias entram no hospedeiro susceptível, se dispersam pelos vasos linfáticos e sanguíneos para alcançar células endoteliais, as quais possuem especial tropismo, causando vasculite (74,75). As manifestações como sinais

clínicos e sintomas de adoecimento apresentam-se entre 2º a 14º dias após a infecção (25,26).

A FM é uma doença sistêmica com sinais clínicos e sintomas: febre, cefaleia, mialgia, mal-estar generalizado, náuseas e vômitos, facilmente confundidos com outras doenças. Além disto, poucos pacientes apresentam exantema, que poderia ser um melhor indicador de FM (26,76,3). Se não for rapidamente instituído tratamento, o paciente evolui para infecção generalizada, com complicações pulmonares, vasculares, desidratação, choque, coma e morte (26,77,78).

A alta letalidade pode estar associada à demora na suspeição clínica da doença, pela ausência de sinais específicos, o que dificulta o diagnóstico diferencial e consequentemente, ausência ou início inoportuno de tratamento adequado (76), além do desconhecimento da doença por parte de agentes de saúde, falta de experiência médica e/ou tratamentos específicos (9,26).

1.4 Diagnóstico Laboratorial

Embora existam diferentes técnicas laboratoriais: Sorologia, Reação em Cadeia da Polimerase (PCR), Isolamento, Imunohistoquímica e Histopatologia (79), as duas primeiras são de uso geral no diagnóstico e confirmação de casos humanos de FM.

A Reação de Imunofluorescência Indireta (RIFI) é o método sorológico padrão ouro para detecção da infecção humana com *Rickettsia* do GFM (25,26).

Em geral, os anticorpos são detectados a partir do 7º até o 10º dia da doença. Os anticorpos IgM podem apresentar reação cruzada com outras doenças (dengue, leptospirose, entre outras) e por isso na interpretação diagnóstica são utilizados os títulos de IgG, que aparecem pouco tempo depois dos IgM e são os mais específicos e indicados para a interpretação diagnóstica (25,26,80). A presença de um aumento de quatro vezes nos títulos de anticorpos, observado em amostras pareadas de soro, é o requisito para confirmação diagnóstica pela sorologia (26,81). Toda a análise do resultado laboratorial deve ser interpretada dentro de um contexto clínico e epidemiológico (26,81).

1.5 Tratamento

O tratamento adequado e oportuno com doxicilinas ou cloranfenicol das infecções humanas com *Rickettsia,* nos primeiros cinco dias da evolução da doença FM, é a garantia de evitar pacientes mórbidos e na redução das taxas de letalidade (26,81,82). Outros trabalhos descrevem quadros de tratamentos para diferentes grupos etários e estados fisiológicos, empregando diferentes antibióticos: Claritromicina, Azitromicina e Josamicina (13,83)

Em pacientes com suspeita clínica, antecedência de picada de carrapato ou procedência de áreas endêmicas para FM, recomenda-se o tratamento precoce, ainda com a ausência de provas laboratoriais (26,80).

1.6 Vigilância

Nos últimos dez anos, a epidemiologia da FM no Brasil foi marcadamente influenciada por quatro ações em saúde: inicialmente pela Portaria MS nº 1.943, de 18/10/2001(10) onde a FMB passa a ser de notificação compulsória. Entretanto, apesar do histórico de casos e sabida existência de áreas endêmicas para FM no RJ, somente dois anos depois da publicação desta portaria, é que se confirmam os primeiros casos no RJ.

A segunda ação acontece no ano de 2007, quando a doença passou a integrar o Sistema de Informação de Agravos de Notificação, na versão Sinan-Net (25), correspondente à mudança no sistema de vigilância, também se inserem novas variáveis, especialmente a evolução do caso, podendo evidenciar as taxas de letalidade e se definem claramente os critérios de diagnósticos de casos.

A terceira ação se dá, a partir do ano de 2011, com o início da capacitação na vigilância de ambiente das rickettsioses promovida pela Secretaria de Vigilância em Saúde (SVS/MS), e pelo Laboratório de Referência Nacional em Vetores das Riquetsioses (LIRN-IOC/FIOCRUZ), em todo o território brasileiro, evidenciando o significativo aumento da atividade de vigilância ambiental para FM no país (84) .

A quarta ação surge com a Portaria Nº 1.271, de 6 de Junho de 2014 (26) onde a FM e outras rickettsioses passaram a compor as doenças de notificação compulsória em até 24 (vinte e quatro) horas, a partir do conhecimento da ocorrência de doença, agravo ou evento de Saúde Pública, pelo meio de comunicação mais rápido disponível, às três esferas do Sistema Único de Saúde (SUS).

1.7 Prevenção

As medidas preventivas são o pilar para evitar ser infestado por carrapatos, adoecer e morrer pelos patógenos que eles podem transmitir.

O humano é hospedeiro acidental dos carrapatos. Ele se infesta no contacto com hospedeiros naturais ou ao entrar em áreas infestadas por carrapatos. Portanto, evitar o contato com carrapato é medida eficaz de prevenção à FM.

Nas áreas onde há trânsito de animais, como trilhas, coleções hídricas, comedouro, etc., é recomendado o reconhecimento rápido da área à procura de carrapatos, bem como fazer vistorias periódicas nas vestimentas para identificar precocemente a infestação por carrapato (81)

Nas áreas de confinamento de bovino, equinos e cães ou áreas silvestres de pesca ou qualquer outra atividade de campo, recomenda-se a utilização de roupas claras que facilitem a visualização dos carrapatos (34,85). Nas áreas do corpo expostas, podem ser usados repelentes (38,81)

No ambiente doméstico, os animais sinantrópicos podem manter populações de carrapatos e disseminá-los no peridomicílio (especialmente nas áreas com pasto) ou bem mantê-los no intradomicílio. Nesse sentido, medidas sanitárias de higienização do ambiente, como o capina periódica da vegetação, e cuidado dos animais de estimação (banho com carrapaticidas), devem ser tomadas(26,3,81)

Para pessoas que trabalham em locais onde risco de infecção é eminente, como, por exemplo, áreas reconhecidamente endêmicas da doença, ou que sua atividade ocupacional oferece risco de infecção (biólogos, médicos veterinários, agricultores,

tratadores de animais), é imprescindível o uso de equipamentos de proteção individual (86). Para evitar o deslocamento acidental de carrapatos, das áreas de alto risco, recomenda-se também que ao sair da área efetue-se uma vistoria minuciosa no corpo e troca das vestimentas (15).

1.8 Controle

As ações de controle devem sempre estar voltadas na eliminação das populações de carrapatos em ambientes de visitas frequente de humanos (parques públicos, zoológicos, etc.). Como a FM envolve ciclos silvestre, peridoméstico e doméstico, com ampla heterogeneidade de hospedeiros e carrapatos, o controle da doença é uma estratégia que deve ser analisada com bastante critério e orientada por um profissional capacitado (34,82). As recomendações oficiais estão pautadas no controle do vetor somente em hospedeiros domésticos como cães, cavalos e outros animais de criação e no manejo da vegetação (26,81,84).

Alguns estudos têm demonstrado que a redução das áreas com pasto "sujo" por meio da capina, interfere no microclima que é necessário para as fases de vida livre do carrapato, impactando favoravelmente na redução das taxas de infestação de ambientes (37,65,3). No entanto, sua aplicabilidade em áreas de mata ciliar ou áreas de preservação é impossível (37). Não é recomendado o uso de produtos químicos para o controle de vetores em ambientes naturais próximos a recursos hídricos.

É importante salientar que qualquer programa de controle de carrapatos deve ser considerado como um programa contínuo, com resultados que serão

evidenciados somente a médio ou a longo prazo (37). Dada essas particularidades, a principal estratégia de controle da FM está pautada ainda na educação em saúde, sendo de extrema importância a divulgação da presença do vetor e da circulação da doença e das medidas preventivas contra a infecção (26,3,86).

1.9 Vulnerabilidade

O conceito de vulnerabilidade considera a contextualização de um evento dentro do universo biológico e social. Assim, Sant'Anna e Hennington (87) reconhecem a vulnerabilidade diante de um agravo ou doença como a compreensão integrada dos riscos, fazendo emergir, simultaneamente, questões éticas, políticas e técnicas que conformam a distribuição dos riscos nos territórios e a capacidade das populações de enfrentá-los. Assim, o estabelecimento do caso humano de FM está relacionado às condições de vulnerabilidade do ambiente (22,3), vulnerabilidade sociodemográfica e à disponibilidade ou carência de recursos destinados à proteção pessoal (88).

Poderíamos ponderar que poucas espécies de carrapatos consideradas reservatórios e vetores de *Rickettsia* patogênicas e alguns mamíferos amplificadores e reservatórios deste bioagente, são elementos primários pela ocorrência e manutenção dos ciclos epizoóticos e na emergência e/ou reemergência de surtos epidêmicos acidentais da FM, RJ. Embora, sejam os determinantes principais dos ciclos de transmissão das bactérias, não são fatores de perigos suficientes pelo estabelecimento de área de risco, vulnerabilidade e estabelecimento de programas de vigilância e controle de surtos humanos, como se

demonstra com os achados de diferentes trabalhos (58,59,86,89–91). Primeiro, porque a ecologia das populações dos carrapatos além de ser influenciada por fatores bióticos (hospedeiros, predadores, tipo de vegetação, fragmentação do habitat, perdidas ou migração de animais, etc), também é afetada por fatores abióticos (temperatura, humidade, precipitações, altitude, etc) (38,58–60,57,61–65); com isso, são fundamentais na variação espaço-temporal no risco de transmissão de patógenos aos humanos (66–68).

2   OBJETIVOS

Objetivo Geral

Analisar a situação ecoepidemiológica e vulnerabilidade espacial da Febre Maculosa no Estado do Rio de Janeiro

Objetivos Específicos

Determinar os atributos ecológicos (hospedeiros, carrapatos e *Rickettsia*) e sua relação com os casos de Febre Maculosa no Estado do Rio de Janeiro.

Analisar o desempenho do sistema de vigilância epidemiológico na notificação e confirmação de casos suspeitos de Febre Maculosa no Estado do Rio de Janeiro

Determinar a vulnerabilidade espacial de infecção humana com rickettsias patogênicas transmitidas por carrapatos no Estado do Rio de Janeiro

3 MATERIAL E MÉTODOS

3.1 Área de Estudo

O Estado do Rio de Janeiro está localizado na porção leste da região Sudeste do Brasil, ocupando uma área de 43.777,954 km^2 e está dividido em 92 municípios (**Figura 1**). É o quarto menor estado do Brasil. Possui a maior densidade demográfica (365,23 hab/km^2) com população estimada em 16.635.996 habitantes e é o estado mais urbanizado do país, com 97 % da população morando em cidades (92).
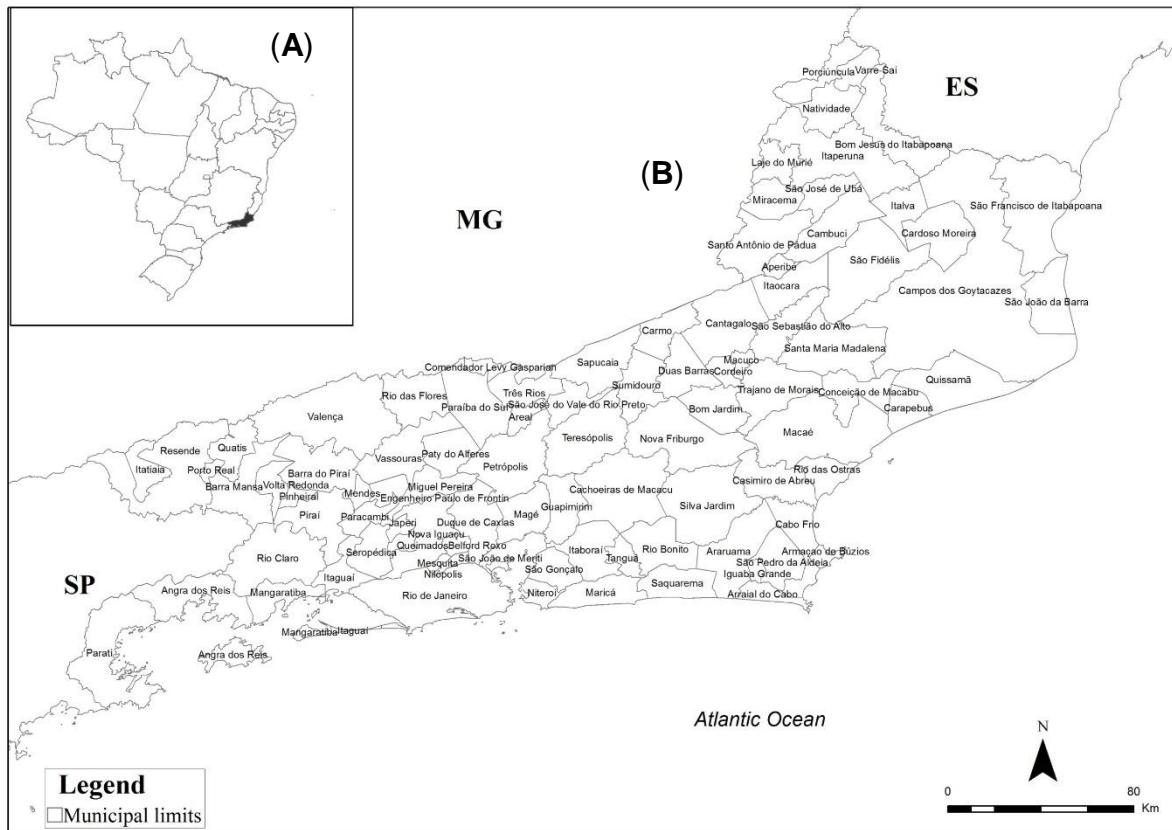
Figure 1. Localização do Estado do Rio de Janeiro, Brasil, (**A**) e municípios do Estado (**B**).

3.2 Dados Epidemiológicos

As informações epidemiológicas provêm de dados não publicados da Secretaria de Estado de Saúde do Rio de Janeiro (SES/RJ), correspondendo ao período de 1980 até 2000 e de dados disponíveis no SINAN decorrentes dos anos de 2001 até 2016. Esses dados foram disponibilizados de forma a proteger a identidade dos pacientes e, portanto, em nenhum momento são apresentadas informações como o nome dos indivíduos ou endereços.

3.3 Dados dos Ectoparasitos

Esses dados foram obtidos através da análise dos laudos emitidos pelo Laboratório de Referência Nacional em Vetores das Riquetsioses - LIRN, Instituto Oswaldo Cruz (FIOCRUZ), de amostras provenientes de vigilância e investigação de casos de FM no RJ, realizadas pela equipe de Saúde Estadual (GPA-LACEN, RJ) no período de 2004 até 2016.

As unidades amostrais de coleta dos ectoparasitos foram constituídas por espécimes oriundos de um mesmo hospedeiro ou ambiente, que passaram a ser relacionados para análises de associação com seus respectivos ectoparasitos. Os ectoparasitos coletados foram identificados usando-se chaves dicotômicas específicas (93–95).

Os artrópodes foram submetidos à extração de DNA (Aljanabi & Martinez 1997). E, para a pesquisa de genes de rickettsias, realizada através da PCR, as

amostras foram definidas por espécime único ou reunidos em grupo (*pool*), segundo o hospedeiro/ambiente, data de coleta, estádio e/ou sexo.

Na PCR, foram utilizados *primers* gênero-específicos (*glt*A) para detecção de *Rickettsia* spp. e *primers* grupo-específicos, para detecção de RGFM (*omp*A) (96). O preparo das soluções e as condições das corridas da PCR foram adequados para cada tipo de gene pesquisado. Para a visualização dos fragmentos de DNA amplificados, as amostras foram submetidas à eletroforese em gel de agarose a 2 %, coradas por Brometo de Etídio e observadas em *scanner* de gel com luz de ultravioleta (97).

3.4 Métodos

As técnicas e métodos de análises variaram segundo os artigos ou produtos obtidos (**Vide o item 4**). Em termos geral, os dados epidemiológicos foram processados para duas análises: uma descritiva e outra analítica. Na primeira, foram depurados os dados segundo duplicidade e inconsistência na informação dos casos, separados entre casos notificados, confirmados e óbitos. Análises de frequência foram feitas segundo as variáveis epidemiológicas (tempo, pessoa e lugar): gênero, área de procedência (urbana, periurbana e rural), sinais e sintomas clínicos, provas laboratoriais e conclusão do caso. A taxa de letalidade (relação entre o número de óbitos pelo agravo e o número de pessoas que foram acometidas pela doença) foi calculada a partir do número absoluto de óbitos pelo número de casos confirmados por ano e município do RJ.

Nas análises analíticas foram empregadas técnicas de estatística multivariada em programas estatísticos (98,99), cartográficas por pacotes TerraView e ArcGis 10.4v (100,101) e de inteligência de máquinas (102,103) para compreensão da epidemiologia e associação com os carrapatos e o espaço geográfico.

4   RESULTADOS

Capitulo 1. Spotted Fever: Epidemiology and Vector-*Rickettsia*-Host Relationship in Rio de Janeiro State

Corresponde ao objetivo específico 1

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Spotted Fever: Epidemiology and Vector-*Rickettsia*-Host Relationship in Rio de Janeiro State

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The eco-epidemiological scenario of spotted fever (SF), a tick-borne disease that affects humans and other animals in several countries around the world, was analyzed in Rio de Janeiro (RJ) State, Brazil. During the last 34 years, 990 SF cases were reported in RJ (the Brazilian state with the highest population density), including 116 cases confirmed by serology (RIFI) or PCR, among 42.39% of the municipalities with reported cases of SF. The epidemiologic dynamics of SF in RJ State are very heterogeneous in time and space, with outbreaks, high mortality rates and periods of epidemiological silence (no SF cases reported). Furthermore, it exhibited a changing epidemiological profile from being rural to becoming an urban disease. This study identified arthropods infected with *Rickettsia felis*, *R. bellii* and *R. rickettsii*, and found that the abundance of ectoparasites was associated with specific hosts. The *R. rickettsii*-vector-host relationship was most evident in species-specific parasitism. This suggests that the association between dogs, cattle, horses, capybaras and their main ectoparasites, *Rhipicephalus sanguineus* and *Ctenocephalides felis*, *Rhipicephalus microplus*, *Dermacentor nitens*, and *Amblyomma dubitatum*, respectively, has a key role in the dynamics of *R. rickettsii* transmission in enzootic cycles and the maintenance of carrier ectoparasites, thus facilitating the existence of endemic areas with the ability to produce epidemic outbreaks of SF in RJ. This study found confirmed human infections for only the *R. rickettsii* carrier *Amblyomma sculptum*, which reinforces the importance of this species as a vector of the pathogen in Brazil. This study can be adapted to different eco-epidemiological scenarios of spotted fever throughout the Americas.

Keywords: public health, eco-epidemiology, rickettsioses, tick-borne diseases, zoonosis

INTRODUCTION

Rickettsioses are diseases caused by obligate intracellular bacteria of the genus *Rickettsia*. Different species of the Spotted-Fever Group of *Rickettsia* (SFGR) are considered etiologic agents of spotted fever (SF), a zoonosis widely distributed throughout the world, with seasonal and sporadic outbreaks that may involve high mortality rates (Raoult and Roux, 1997; Rudakov et al., 2003; Parola et al., 2009, 2013; Eremeeva and Dasch, 2015).

In nature, the SFGR transmission-cycle is maintained by the capacity of ticks to act as vectors, reservoirs and/or amplifiers of the bacteria. Ticks can remain infected throughout their lives through transovarial and/or transstadial transmission. The continued presence of the bacteria in a population may also be the result of ticks obtaining a blood meal, and transmitting the bacteria to a wide variety of mammals, including humans (Dumler and Steohen, 2005; Parola et al., 2013; Eremeeva and Dasch, 2015).

Spotted fever is the most prevalent tick-borne illness in Brazil (Fiol et al., 2010; Brasil, 2014a), having been first reported in the country in the early 20th century (Fialho, 1929; Piza et al., 1931). A variety of species of *Rickettsia*, and their vectors, have since been identified in areas with reported cases of SF (Cunha et al., 2009; Gehrke et al., 2009; Medeiros et al., 2011; Szabó et al., 2013; Moura-Martiniano et al., 2014), illustrating the complexity of its enzootic and epidemic cycles, as well as the diversity of potential vectors.

There have been confirmed cases of SF in 21 out of 23 Brazilian states (Brasil, 2015a). Morbidity is moderate, but the lethality rate is high, exceeding 80% in severe cases (Szabó et al., 2013; Brasil, 2014a), when the rash becomes petechial and then hemorrhagic, consisting mainly of bruises or suffusions (Brasil, 2014a).

The Southeast Region of Brazil has the greatest number of confirmed cases of, and the most deaths by, SF (Brasil, 2015a), with outbreaks in different eco-epidemiological scenarios (Medeiros et al., 2011, Szabó et al., 2013, Moura-Martiniano et al., 2014; Nasser et al., 2015). Furthermore, within this region, the geopolitical space of Rio de Janeiro (RJ) State stands out as having one of the highest population densities in the country, experiences great tourism and possesses large urban and rural areas in different ecoregions.

Although some studies on SF in the Americas have addressed a holistic view of the ecological components associated with the epidemiological dynamics of the disease (Labruna, 2009; Parola et al., 2009; Eremeeva, 2012; Quintero et al., 2013; Szabó et al., 2013), studies in endemic areas are scarce. Therefore, the present study aims to analyze the vector-*Rickettsia*-host relationship in RJ and its relation to

FIGURE 1 | (A) Map of Brazil highlighting the location of Rio de Janeiro State. **(B)** Health division of Rio de Janeiro State, Brazil, indicating the municipalities that contributed to the analyzed data. Source: Secretaria de Estado de Saúde do Rio de Janeiro- http://www.saude.rj.gov.br/.

FIGURE 2 | Reported cases (A), confirmed cases **(B)**, and deaths **(C)** from spotted fever in municipalities of Rio de Janeiro State in two study periods.

the dynamics of SF cases from the 1980s to 2014, in order to assess the factors that are associated with SF in RJ.

MATERIALS AND METHODS

Study Area

Located in the eastern portion of the Southeast Region of Brazil, RJ) occupies an area of 43,777.954 km^2, and is comprised of 92 municipalities (**Figure 1**). It has the largest population density in Brazil (365.23 inhabitants/km^2), with an estimated population, in 2015, of 16,550,024 inhabitants. It is the most urbanized region

of the country, with 97% of the population living in cities (IBGE, 2015).

Epidemiological Data

Epidemiological data were obtained from the Secretary of State of Health of RJ (SES/RJ) and from the Sistema de Informação de Agravos de Notificação (SINAN) (Brasil, 2015b), for the time frame from 1980 to 2014. The data were chronologically divided into two periods: Period 1 corresponding to the years from 1980 to 2004; and Period 2 corresponding to the years from 2005 to 2014. This separation of data was related to the epidemiological dynamics of reported cases. In the second period, epidemiological descriptive data (time, place and person) are included in SINAN reporting forms.

Putative Vectors of SF

Information regarding potential vectors of SF was obtained from the database of the Laboratório de Referência Nacional em Vetores das Riquetsioses (LIRN; Labortory of the National Reference of Rickettsial Vectors), built from samples analyzed and received within the flow of the Rede Nacional de Vigilância de Ambiental para Febre Maculosa e outras Rickettsioses (National Network for Environmental Monitoring for Spotted Fever and other Rickettsial Diseases), of Ministry of Health from 2005 to 2014. The samples were collected in sampling units (specimens originating from the same host or environment, collected on the same date and during the same epidemiological outbreaks) by state and municipal health teams from RJ for environmental monitoring and investigating cases of SF.

In general, the samples were collected by cloth-dragging, visual searches on hosts and inspection of plant litter and abiotic surfaces.

Ticks were identified using dichotomous keys for larvae (Amorim and Serra-Freire, 1999), nymphs (Martins et al., 2010), and adults (Aragão and da Fonseca, 1961; Barros-Battesti et al., 2006), or specific descriptions (Maroun et al., 1999; Walker, 2000). Fleas were identified according to Linardi and Guimarães (2000), and lice according to Ferris (1951) and Price et al. (2004).

Voucher specimens were deposited in Coleção de Artrópodes Vetores Ápteros de Importância em Saúde das Comunidades – CAVAISC/FIOCRUZ, while the remaining specimens were submitted to total DNA extraction by salt-extraction technique (Aljanabi and Martinez, 1997). Species of *Rickettsia* were identified using PCR. In this process, samples were analyzed as single or pooled specimens ranging from 2 to 10 individuals of the same sampling units, stage of development (larva, nymph, and adult), and sex. For PCR, we used *Rickettsia* genus-specific primers targeting the *gltA* gene (CS4 239/CS4 1069, CS2 78/CS2 323) (Labruna et al., 2004) and SFG-specific primers targeting the *ompA* gene (*Rr* 190.70p/ *Rr* 190.602n) (Regnery et al., 1991).

Amplified DNA fragments were visualized using 2% agarose gel stained with ethidium bromide and observed using a gel scanner with ultraviolet light (Sambrook and Russell, 2001).

Previous research (Gehrke, 2010; Moura-Martiniano et al., 2014) used ectoparasites from SF research and surveillance areas in RJ, collected throughout 2005–2009. In these studies, amplicons that corresponded to the expected amplified size

FIGURE 3 | Epidemiological dynamics of spotted fever in Rio de Janeiro State, from the 1980s to 2014.
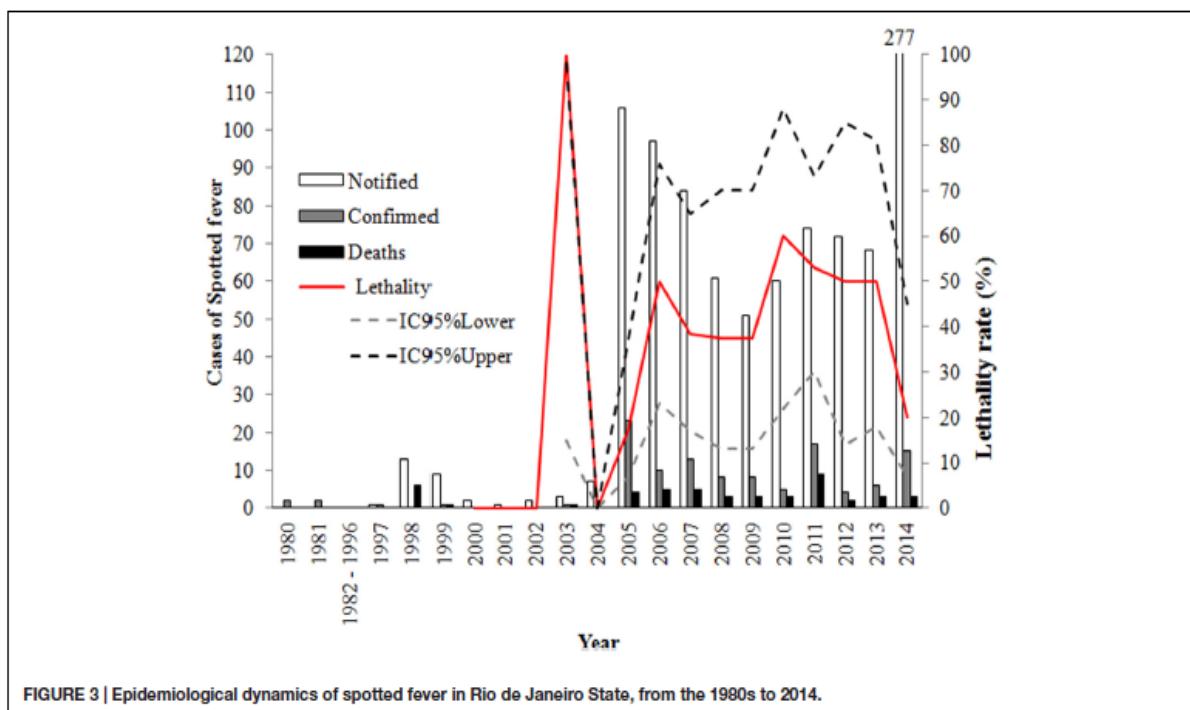
were purified and sequenced. The resulting sequences were identified by comparison with the GenBank database using BLAST. These sequences were also submitted to phylogenetic reconstruction. All obtained sequences were deposited in GenBank (Supplementary Table 1).

Data Analysis

All information obtained was tabulated according to the type of origin (investigation of cases or regular environment surveillance of SF) and submitted to descriptive statistical analysis; frequency measures, in the case of epidemiological data; and analyses of the percentage of similarity – SIMPER (Clarke, 1993) for biological data. SIMPER was used to highlight species with the highest contribution to abundance among the municipalities of RJ using standardized data of richness and abundance of vectors and the association measure of Bray–Curtis using the statistical package Plymouth Routines In Multivariate Ecological Research – PRIMER-v.5 (Clarke and Gorley, 2006).

To identify statistical patterns of association between parasites (dependent variable) and hosts (independent variable), biological data determined most representative by SIMPER analyses were used for exploratory non-metric multi-dimensional scaling (NMMDS) (Clarke, 1993) using PRIMER-E. Data were standardized with an association measure of Bray–Curtis. In these analyses, the *Bubble* value of each association is graphically represented on a Cartesian plane by the size (number of individuals) and frequency (number of samples) of each species of ectoparasite collected from each host. For visual comparisons, Bubble groups of each analysis were allocated in the same plane

by using ruler measurements and the line grid of the Paint program of Microsoft Office package.

Using the program TerraView (INPA, 2010), and data by area, cartographic analyses were performed using epidemiological thematic maps, with vectors and natural infection with Rickettsial genes to determine distribution, probabilistic patterns and the relationship between these variables using kernel density estimators.

RESULTS

Epidemiology

Nine hundred and ninety suspected SF cases were reported during the study period in 73.91% (68/92) of the municipalities within the study area. Of the 990 cases, 11.71% (116/990) were confirmed, including patients residing in 42.30% (39/92) of the municipalities with reported SF cases (**Figures 2A,B**). Five municipalities (Barra do Pirai, Paraty, Petrópolis, Portiuncula, and Rio de Janeiro) possessed 52.59% (61/116) of the confirmed cases. The municipality of Rio de Janeiro, the State's capital, had the highest rate, with 18.97% (22/116) of the cases (**Figure 2B**). In 64.10% (25/39) of the municipalities with confirmed cases, there were 48 deaths (**Figure 2C**).

The epidemiological dynamics of SF in the study area shows chronologically different profiles (**Figure 3**). In the first period, between 1980 and 2004, records were mainly quantitative with little additional information. During this period, there were 41 reported cases, with 9 being confirmed and 14 reported deaths.

In the second period, between 2005 and 2014, 949 cases were reported, with 28.11% (107/949) being confirmed and 37.38%

FIGURE 4 | Rickettsial vectors modeled by their hosts in Rio de Janeiro State, from 2005 to 2014. (A) During investigation of spotted fever cases, **(B)** In environment surveillance.
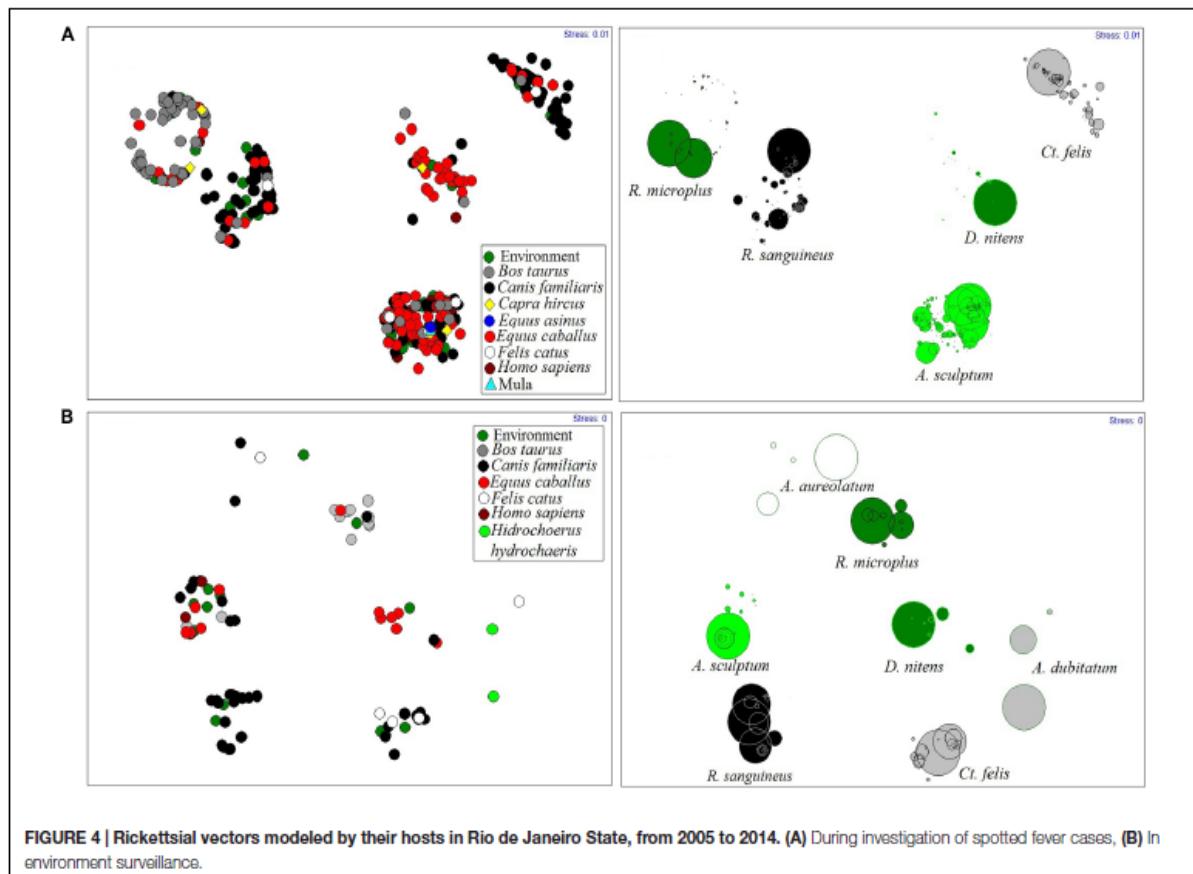
(40/107) advancing to death. Confirmed cases were distributed throughout the year, with higher frequencies during the months of July to October. The reported cases of this period also possessed a greater amount of descriptive data. From 2007 to 2014, in 28.95% (22/76) of the cases, the infection occurred in an urban area, 13.15% (10/76) in a peri-urban area and 35.53% (27/76) in a rural area, with the area of infection being unknown in 22.37% (17/76) of the cases.

Households infections occurred in 27.63% (21/76) of the cases, while 22.37% (17/76) occurred during leisure activities and 17.11% (13/76) during labor activity, while 32.89% were not specified.

Males were more frequently infected than females, accounting for 61.84% (47/76) of the infections. In terms of age, the population of reproductive age and the work force, between 20 and 59 years, was the most compromised (57.33%) age group. Even within that period (2007–2014), we found eight cases of SF caused by *R. rickettsii* (five turned to death, one progressed to healing and two ignored) and one case caused by *R. parkeri* (progressing to cure).

Richness and Abundance of Vectors

Distributed among 650 samples, 8,064 specimens of 14 species of wingless arthropods of interest to public and animal health were identified.

Ticks (Acari: Ixodidae) had the greatest abundance and species richness. In order of contribution, the species are: *Amblyomma sculptum, Rhipicephalus sanguineus, Rhipicephalus microplus, Dermacentor nitens, Amblyomma aureolatum, Amblyomma dubitatum, Ornithodoros* spp., *Amblyomma ovale, Haemaphysalis leporispalustris* and *Amblyomma longirostre* (data not shown). The representation of Insecta was dominated by the flea *Ctenocephalides felis*, with *Polygenis* spp, *Ctenocephalides canis* occurring in very low frequency, and the lice *Felicola felis* and *Pediculus humanus* occurring in even lower frequency (data not shown).

Of the 37 municipalities with samples sent to LIRN, 30 occurred during the investigation of cases, 10 of which were also part of regular environment surveillance of SF. Seven municipalities had information only for environmental monitoring.

According to SIMPER analysis, the structure of the community of arthropod-carriers of *Rickettsia* spp. is heterogeneous in the study area, and independent of the origin of the samples (investigation or monitoring). Four species of ticks (*A. sculptum, Rh. sanguineus, Rh. Microplu* and *D. nitens*) and one flea (*Ct. felis*) contributed to 99% of the total arthropod-carrier abundance. However, the contribution of *A. sculptum* to total relative abundance was dominant in both

foci and environmental monitoring areas with 78.67 and 48.86%, respectively.

Vector-Host Relationship

Potential *Rickettsia* vectors were found parasitizing eight mammalian species, *Bos taurus* (cattle), *Canis familiaris* (dog), *Capra hircus* (sheep), *Equus asinus* (ass) *Equus caballus* (horse), *Felis catus* (cat), *Hidrochoerus hidrochaeris* (capybara) and *Homo sapiens* (human); and two morphospecies Mula and *Bos* spp. In the environment, mainly immature stages (larvae and nymphs) of *A. sculptum* and *Rh. microplus* were found.

Using the five species that contributed 99% of the total abundance, the NMMDS analyses, with stress values in 2D and 3D of 0, found no statistically significant spatial distribution model for vectors in municipalities. With the same amount of stress, NMMDS found that the distribution of ectoparasites to be modeled by their hosts (**Figure 4**).

Except for *A. sculptum*, in areas under investigation or surveillance, most species were associated with a single host. Thus, for example, the horse is responsible for maintaining the populations of *D. nitens*, *B. taurus* modeled populations of *Rh. microplus* and the dog was the host most parasitized by *Rh. sanguineus* (**Figure 4**).

Vector-Pathogen-Host Relationship

The presence of the *glt*A gene was detected in 26 samples of ticks and 24 samples of fleas. Yet, in 43 samples of ticks and 15 of fleas the *ompA* gene was amplified. Sequences obtained from some samples of RJ ectoparasites exhibited identities of between 97 and 100% with *R. rickettsii* and *R. felis* (**Table 1** and Supplementary Table 1). In those species in which *R. rickettsii* genes were found, the ectoparasites had been collected on their specific hosts. *A. sculptum* was the only species found parasitizing humans with the presence of genes of the causative agent of Brazilian spotted fever (**Table 1**).

Probabilistic analyses of the distribution of the species *A. sculptum*, *Rh. sanguineus* and *Ct. felis*, with the presence of genes for SFGR and *R. rickettsii*, had the same spatial model as the likelihood of cases and deaths in the SF study area (**Figure 5**). The likelihood of cases and deaths by SF was found to be higher in the municipalities of Metropolitan, Middle Paraíba and Northwest Regions of RJ (**Figure 5**).

DISCUSSION

The first reports of SF in the study area were in the 1940's, and it was considered a disease of rural areas for several decades (Tostes and Bretz, 1941; Greca et al., 2008). More recently the profile of SF has changed, with cases of infection reported for urban and peri-urban areas between 2007 and 2014 outweighing cases in rural areas because of the greater population density. However, the process of SF urbanization does not seem to be common throughout Brazil (Barros-Silva et al., 2014), but it is evident in the southeastern part of the country (Nasser et al., 2015; Souza et al., 2015). Some studies have reinforced the importance of areas experiencing urban expansion and deforestation, or places

TABLE 1 | Vector/carrier-*Rickettsia*-host relationship in Rio de Janeiro State, 2005–2014.

Host\Vector	Amblyomma sculptum	Rhipicephalus sanguineus	Ctenocephalides felis	Ctenocephalides canis	Rhipicephalus microplus	Dermacentor nitens	Amblyomma spp.	Amblyomma aureolatum	Amblyomma dubitatum
Environmental	R	R, R-GSF	R. rick, R. fe		*	*	*	*	*
Bos taurus	R, R-GSF				R, R-GSF, R. rick				
Canis familiares	R, R-GSF, R. rick	R, R-GSF, R. rick	R, R-GSF, R. fe, R. rick	R. rick	*		R	R	
Capra hircus	*								
Equus asinus	*								
Equus caballus	R, R-GSF, R. rick		R, R-GSF, R. fe, R. rick		R-GSF	R, R-GSF, R. rick			
Felis catus	*		R, R-GSF, R. fe, R. rick			*		*	*
Hydrochoerus hydrochaeris							*		
Homo sapiens	R-GSF, R. rick				*				
Mula									R. rick, R. belli

R, *Rickettsia*; R-SFG, *Rickettsia* of Spotted fever group; R. rick, *Rickettsia rickettsii*; R. fe, *Rickettsia felis*. * Infestation without detection of *Rickettsia*.
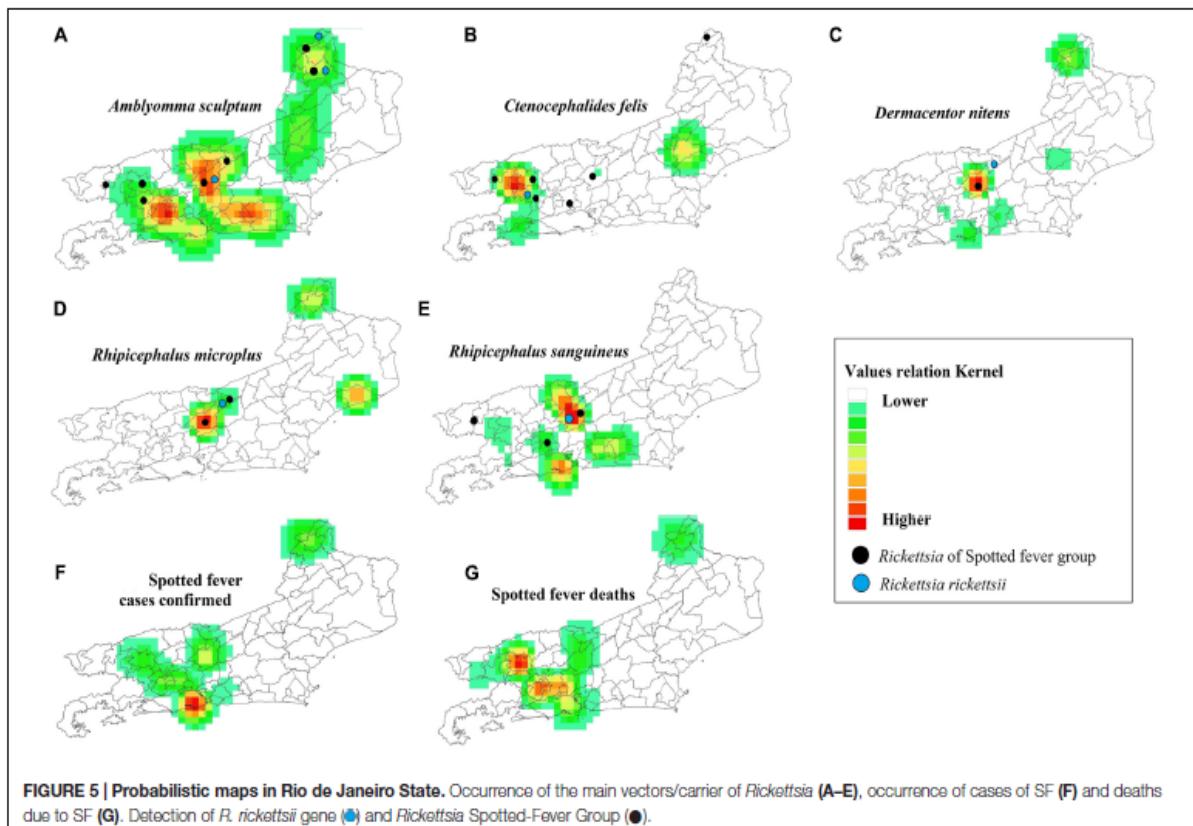
FIGURE 5 | Probabilistic maps in Rio de Janeiro State. Occurrence of the main vectors/carrier of *Rickettsia* **(A–E)**, occurrence of cases of SF **(F)** and deaths due to SF **(G)**. Detection of *R. rickettsii* gene (●) and *Rickettsia* Spotted-Fever Group (●).

where work is done with mammals in confinement, and other environmental determinants in the epidemiological dynamics of SF (Gazeta et al., 2009; Labruna, 2009; Ogrzewalska et al., 2011; Moura-Martiniano et al., 2014; Rozental et al., 2014; Nasser et al., 2015). In fact, regions of higher density seem more conducive to the emergence of cases in southeastern Brazil, even in rural areas (Ribeiro et al., 2013).

The observed epidemiological dynamics of SF overtime (**Figure 2**) are probably related to the perception of the disease in the context of varying health policies at different time periods, which seems to have influenced the sensitivity of the health system in detecting cases. Investigations into the sensitivity of the epidemiological surveillance system for the catchment, diagnosis and management of patients with SF are being prepared for publication.

Thus, between 1980 and 2000, SF was only reported for the most morbid cases and mainly during a few recognized outbreaks. As of 2001, reporting of SF cases became mandatory in Brazil (Brasil, 2001), and although there are studies that show cases and deaths in 2001 in RJ (De Lemos et al., 2002), the first confirmed cases of SF appeared in SINAN in 2003.

However, the number of cases increased significantly from 2005 due to wide media exposure of a SF outbreak in the Highland Region of RJ. Similarly, a better definition of the epidemiological profile of SF was integrated into the new notification system in 2007 (Brasil, 2009), when new variables

were included in reports; in particular the evolution of cases, which allows mortality rates to be determined, and clearly defining cases with diagnostic criteria.

Beginning in 2012, with the implementation of the national network for environment surveillance of SF and other Rickettsial diseases in Brazil (de Oliveira et al., 2015), and with Ordinance N° 1.271 (Brasil, 2014b), in which SF and other Rickettsial diseases were included as diseases of immediate compulsory notification, it became evident that some municipalities of RJ actively participated in environmental surveillance and the reporting of suspicious and actual cases, resulting in an increase in the number of municipalities reporting SF and the number of overall reported SF cases (**Figures 1, 5**).

In general, within the study area the disease is first diagnosed as leptospirosis or dengue, and when the case progresses to death, SF is suspected (Lamas et al., 2008; Moliterno, 2009; Monteiro et al., 2014; Rozental et al., 2014).

All these scenarios can be considered important contributors to the dissonant dynamics of confirmed cases and deaths of SF in RJ (**Figures 1, 3B,C**).

Most ectoparasites, excluding *A. sculptum*, parasitize specific hosts, which seems to be common (Serra-Freire and Furlong, 1993; Gazeta et al., 2009; Gehrke et al., 2009). The obtained sequences of *R. rickettsii*, from *glt*A (*Rickettsia*) and *omp*A (SFGR) genes, are prevalent in this particular parasitism, suggesting that the association between vertebrates and their

main ectoparasites plays a key role in the dynamics of *Rickettsia* transmission in enzootic cycles, which provides endemic areas with the opportunity to give rise to outbreaks of SF.

The present study shows a relationship between the spatial distribution of *A. sculptum*, *Rh. sanguineus* and *Ct. felis* infected with cases of SF, although some studies have also identified these species in areas with incidence of SF cases in RJ (Cunha et al., 2009; Gazeta et al., 2009; Gehrke et al., 2009; Moura-Martiniano et al., 2014). However, the first tick species (*A. sculptum*) has been previously incriminated in the transmission of *R. rickettsii* to humans in Brazil (Greca et al., 2008; Brasil, 2009, 2014a; Szabó et al., 2013).

The results of the present study also found parasitism by *A. sculptum* infected with *R. rickettsii* on humans, which confirms the importance of this species as a vector of this SF agent. Therefore, we consider the other species of ticks (*Rh. sanguineus*, *Rh. microplus*, and *D. nitens*) and the flea (*Ct. felis*) found possessing Rickettsial genes, to be of *carrier* status (Estrada-Peña et al., 2013).

Although this study is the first to spatially display SF endemic areas (**Figures 3B, 5F**), occurrence areas of the main hosts, vectors and arthropod carriers of *R. rickettsii* and SFGR (**Figure 5**), it has some limitations that must be addressed: (1) Epidemiologically there may have several mistake that could not be standardized for a retrospective study; (2) In the routine surveillance system of *Rickettsia* vectors with SFGR genes, few samples were diagnosed to species level, which limits their contribution to molecular taxonomy of *Rickettsia*; it does not allow the determination of whether *R. parkeri* is really circulating in RJ or if it can be associated with cases of SF as data from SES/RJ indicate (one case); and (3) There are other factors that may have had an affect on our results, including the sampling effort, the different techniques of collection, the time between the occurrence of an event and its corresponding case study and the lack of information on vectors in municipalities with confirmed cases of SF.

Future research on the spatial distribution of areas of probable infection, molecular taxonomy of *Rickettsia* in the main vectors, serology of the main hosts identified in this study, relationship between tick phenology and environmental (abiotic) features, application of mathematical models to determining tick niches from survey data (Estrada-Peña et al., 2013), are needed for

a better understanding of disease dynamics and vulnerability factors so as to have a more focused perspective on prevention and control by grievance.

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SUPPLEMENTARY MATERIAL

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Capitulo 2. Evaluating the surveillance system for spotted Fever in Brazil Using Machine-learning Techniques

Corresponde ao objetivo especifico 2.

Situação do manuscrito: Publicado em: Frontier in Public Health

Evaluating the surveillance system for spotted Fever in Brazil Using Machine-learning Techniques

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This work analyses the performance of the Brazilian spotted fever (SF) surveillance system in diagnosing and confirming suspected cases in the state of Rio de Janeiro (RJ), from 2007 to 2016 (July) using machine-learning techniques. Of the 890 cases reported to the Disease Notification Information System (SINAN), 11.7% were confirmed as SF, 2.9% as dengue, 1.6% as leptospirosis, and 0.7% as tick bite allergy, with the remainder being diagnosed as other categories (10.5%) or unspecified (72.7%). This study confirms the existence of obstacles in the diagnostic classification of suspected cases of SF by clinical signs and symptoms. Unlike man–capybara contact (1.7% of cases), man–tick contact (71.2%) represents an important risk indicator for SF. The analysis of decision trees highlights some clinical symptoms related to SF patient death or cure, such as: respiratory distress, con-vulsion, shock, petechiae, coma, icterus, and diarrhea. Moreover, cartographic techniques document patient transit between RJ and bordering states and within RJ itself. This work recommends some changes to SINAN that would provide a greater understanding of the dynamics of SF and serve as a model for other endemic areas in Brazil.

Keywords: public health, epidemiology, spotted fever, machine-learning, decision trees, probabilistic neural networks

INTRODUCTION

Rickettsial diseases are zoonoses caused by bacteria of the genus *Rickettsia* that are transmitted mainly by ticks to mammalian hosts and accidentally to humans. The infections produce an acute fever and systemic complications that can lead to patient death if proper treatment is not provided in time (1–3).

In Brazil, the main rickettsiosis is spotted fever (SF), and infections caused by *Rickettsia rickettsii* are considered the most serious. Moreover, other pathogenic *Rickettsia* (*R. parkeri* and *Rickettsia* Atlantic Forest strain) are also reported in the country, although these cases may or may not be confirmed (4, 5).

Spotted fever is a systemic disease with nonspecific signs and symptoms during its early stages. Throughout its course, it can be easily confused with other diseases, but a few patients develop rashes, which is the best clinical indicator (1, 3, 5–7). High lethality seems to be associated with inaccurate clinical suspicion, which affects diagnosis and treatment opportunity (3, 5, 6).

Given this scenario, it is essential to analyze the efficacy of the Sistema de Informação de Agravos de Notificação—SINAN (Disease Notification Information System) in capturing, managing, and

confirming suspected human cases of SF, and for providing information for analysis of its morbidity profile, thus contributing to decision-making at the municipal, state, and federal levels in Brazil.

Evaluation of a surveillance system (SS), such as SINAN, should promote the best use of public health resources by ensur-ing that only important problems are under surveillance, and that the SS operates efficiently. Insofar as possible, the evaluation of a SS should include recommendations for improving quality and efficiency (e.g., eliminating unnecessary duplication; assessing information potential of the included variables). Above all, an evaluation should assess whether a system is serving a useful public health function and meeting its objectives (8).

Therefore, apart from the monitoring system evaluation model proposed by Klaucke et al. (8), it is important to use other tools to identify the strengths and weaknesses of SINAN so that preventive measures can be implemented and improvements can be made in its organization in order to capture, manage, diagnose, and treat in a timely manner suspected cases of SF, and facilitate a reversal in mortality rates of the disease.

The techniques of machine-learning (ML); promise to be use-ful tools for evaluating the accuracy of the SS for SF since they

are better suited to dealing with a large number of variables and performing massive data analyses than a human agent. From this perspective, this paper employs ML techniques, such as data mining and probabilistic neural network analysis combined with geographical information, in order to better understand the SS of SF (SINAN) in the state of Rio de Janeiro.

MATERIALS AND METHODS

Study Area

The state of Rio de Janeiro is located in the eastern portion of Brazil's Southeast Region and occupies an area of 43,777.954 km_2 divided into 92 municipalities (**Figure 1**). It is the fourth smallest state (by area) in Brazil, yet has the highest population density (365.23 inhabitants/km_2) with an estimated population of 16,636,000 inhabitants and is the most urbanized state in the country, with 97% of the population living in cities (9).

Epidemiological Data

The data presented here was obtained from SINAN and provided by the Secretaria de Estado de Saúde do Rio de Janeiro—SES/
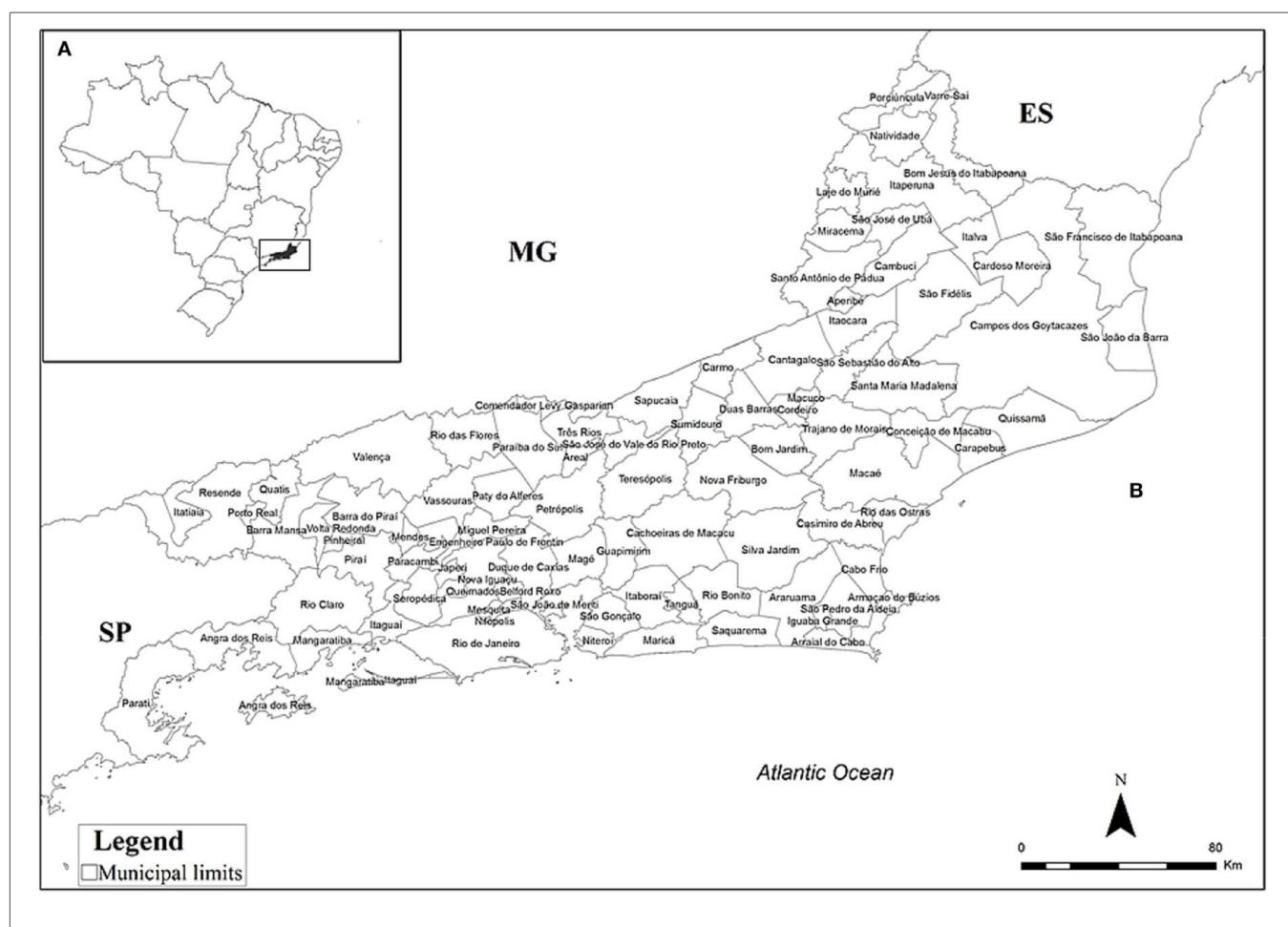
Figure 1 | Location of the state of Rio de Janeiro, Brazil, **(A)** and its municipalities **(B)**. ES, Espírito Santo; MG, Minas Gerais; SP, São Paulo.

36

RJ (State Secretary Health of Rio de Janeiro), and encompassed notifications of suspected cases of SF between 2007 and July 2016. These data were made available with the protection of the identity of the patients; therefore, information such as names or addresses cannot be displayed at any time to comply with national ethical regulations (10).

Although cases reported to SINAN were initially separated into those confirmed by laboratory tests (PCR or Serology) and/ or clinical and epidemiological nexus, unconfirmed cases and ignored cases, as reported in the corresponding epidemiological forms, all were included in the present study.

Methods

Artificial Neural Networks

Classification based on probabilistic neural networks (PNN) (11), which is a feed forward neural network, was the first ML technique implemented for identifying patterns concerning the classification of reported cases into different groups of pathologies. It is a nonparametric method for classifying observations in n groups based on p qualitative and/or quantitative input variables (12–14). It implements a statistical algorithm called Kernel discriminant analysis, whereby, processes are organized to feed forward a multiple network with four types of layers: input layer, pattern layer, addition layer, and output layer (15). Through a ML process, the PNN develops the mathematical ability to perform variable predictions and correctly classify observations within pre-established categories (12–14).

In addition to its advantages over other statistical tests (11, 15), PNN was selected for implementation because of the simple and fast way by which it can process large amounts of information (11, 14, 15), the friendly way the network can be trained and its robustness to noise (14). The PNN has 31 input (p) and 10 output (g) variables. The sample space contains 528 of the 870 cases notified; the others were excluded because they did not contain information of provenance and/or lacked information regarding clinical signs. One hundred and two cases of patient records were selected for training, which contained information on area of residence (urban, peri-urban, and rural) and that confirmed 1 of the following 10 pathological categories (output) for composing the training set, as defined by SINAN: cellulitis, dengue, encepha-litis, hepatitis A, leptospirosis, meningitis, other disease, SF, tick bite allergy, and virosis. The remaining 426 cases were used for testing the neural network. In this scenario, the input layer is composed of 22 clinical variables (fever, headache, abdominal pain, myalgia, nausea, rash, diarrhea, jaundice, hyperemia, hepatomegaly, petechiae, bleeding, lymphadenopathy, convul-sion, necrosis, prostration, shock, coma, hemorrhage, respiratory distress, oliguria, other symptoms), 1 temporal variable (monthly reporting), 7 environmental variables [area of residence, contact with tick, capybara, dog/cat, cattle, horses, nature (forests, rivers, and waterfalls)]; and the variable hospitalization.

All variables except for the month of notification and area of residence were transformed into variables of ternary response (1 = yes or presence, −1 = no or absence, and 0 = no information) to provide values with scales easily comparable to each other. The PNN analyses were done by using the statistical package StatgraphicCenturium XVII (16).

Knowledge Discovery

In this work, we used another ML technique combined with data mining. Briefly, the goal was to automatically build a knowledge representation (17) by using algorithms that process combinatorial searches and discover correlations in large volumes of data. The algorithms used are associated with a technique called decision trees (18), such as: Best First Decision Tree, Decision Stump, Functional Tree, J48, Logistic Model Trees, Reduced-Error Pruning Tree, and Simple Classification and Regression (19, 20). The appropriate algorithm to be used depends on the problem being studied and its constraints, so the algorithm chosen is usually based on literature reports. However, there are no articles describ-ing ML algorithms applied to the problem addressed by the present work. For this reason, an exhaustive test of all listed algorithms was executed. Cross-referencing of 23 clinical and seven epidemiologi-cal variables was performed in order to evaluate if a patient case might prove fatal. Cases in which the evolution was recorded as "ignored" do not contribute positively to the ML process because they introduce a component of uncertainty about the evolution of the case, and so, these cases were excluded from the sample space.

Decision trees were built and optimized using cross-validation over a k number of folds. In such k-fold cross-validation, the original sample is randomly partitioned into k subsamples. Among all k subsamples, a single one is retained as the validation data for testing the model, and the remaining $k − 1$ subsamples are used as training data. The cross validation process is then repeated k times (the folds), with each of the k subsamples used exactly once as the validation data. Then, the k results from the folds are averaged to produce a single estimation. This procedure was accomplished by using the free software Weka (Waikato Environment for Knowledge Analysis) (19).

Mapping Process

The mapping process was performed using the most relevant attributes of the previously discussed analyses and the confirmed cases of SF. The observations of the confirmed cases were studied by measures of central tendency and distribution according to case evolution: recovered, death, and ignored. At this stage, the cases recorded as confirmed by laboratories were compared with the criteria set out in the epidemiological surveillance guides for the years 2007–2016 (4, 5, 21, 22).

Cartographic Techniques

Finally, using the data of confirmed SF cases ($n = 104$), a study of patients spatial behavior was undertaken according to residence, infection, and medical care, using the program Terraview (23). Subsequently, this study was exported to the program ArcGis program (24), which was used to develop thematic maps for the identification of spatial patterns.

RESULTS

Among the 890 SF cases reported in SINAN in RJ, 11.7% (104) were confirmed as SF; 0.7% (6) associated with tick bite allergy; 2.9% (26) as dengue; 1.6% (14) as leptospirosis, and 10.5% (93) as other categories. In addition, 72.7% (647) of reported cases did not have a pathology category provided (**Figure 2**).

37
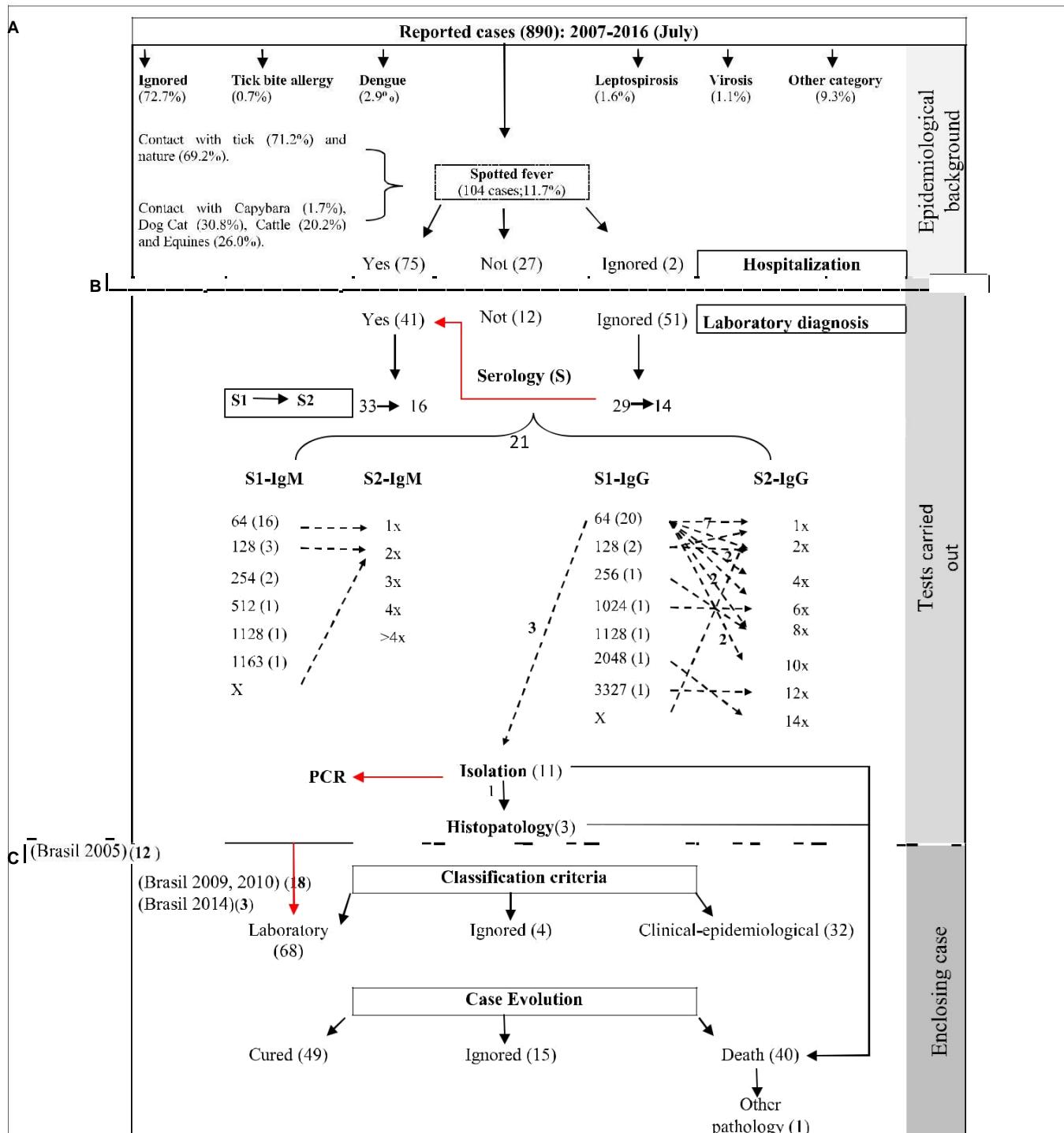
Figure 2 | Process map for epidemiological surveillance of spotted fever (SF), 2007–2016. **(A)** Descriptive epidemiological analysis of the cases reported to SINAN and hospitalization of cases confirmed as SF. Data inconsistency (→). For example, of 51 cases without laboratory tests recorded (ignored), evidence was found in 29 using indirect immunofluorescence assay in the first sample and 14 for the paired sample. **(B)** Follow-up to laboratory techniques and serological titers confirming human cases with SF. Evidence was found for 33 cases through laboratory confirmation following the parameters established for the country (4, 5, 21, 22). Seroconversion serologic titers (→), for example, of 20 patients with IgG titers for 1:64 in the first sample (S1), seven exhibited no increase in titers (1×), two increased by a factor of four (4×), two by a factor of eight (8 ×), and two by a factor of 10 (10×). The zeta no number refers to one seroconversion patient. Serologic titers: 1× = 1:64, 2× = 1:128, 4× = 1:256, 6× = 1:512, 8× = 1:1,024, 10× = 1:2,048, 12× = 1:4,096, 14× = 1:8,192.
  (C) Comparative evaluation of the serological classification criteria with current technical standards (according to period) of Brazil and final clinical evolution of the patients with SF.

About 50% (437) of the reported cases involved hospitalization, but information concerning such hospitalization was available for just 181 patients; that is, there were missing data such as dates of hospitalization and discharge. Among the confirmed SF cases, 75 had been hospitalized, of which, 68 had their diagnosis con-firmed by laboratory techniques and 32 by clinical-epidemiologic criteria; the criterion of classification was not recorded for four of the confirmed cases. Regarding the clinical outcome of the cases, 47.1% (49) of the patients recovered, 38.5% (40) died, and 14.4% (15), there was no information report (**Figure 2**).

Among the clinical signs and symptoms, fever was present in 91.3% (95) of the confirmed cases, followed by headache, myalgia, prostration, and nausea/vomiting. The proportion of the symptoms remained relatively invariant among cases that turned into death, cases that were cured, and cases that were ignored (**Figure 3**).

The neural network was able to classify 38.2% (39/102) of cor-rect instances of diagnosis (**Table 1**). Observe that the probabilis-tic bid for choosing the correct diagnosis is 10.0% since there are 10 possibilities of diseases. Although the 38.2% hit is higher than such probabilistic bid, it is still a poor classifier for determining the nature and circumstances of a diseased condition. Therefore, the PNN failed to produce good agreement in classifying cases into the pre-established disease categories using clinical and predictive environmental variables. It was observed that the Field 51 from SINAM form for recording the diagnosis was frequently not filled properly, and thus there is a lack of information. Consequently, a reduced sample was used for training the PNN (102 cases), which compromised the performance of the neural network, resulting in a low overall percentage of correct classification.

In the analysis of clinical evolution of patients using data min-ing and ML, some of the algorithms had irrelevant results; the best results were obtained with the algorithms Best First Decision Tree, J48, and Reduced-Error Pruning Tree. All of the algorithms generated decision trees for identifying probable deaths with only epidemiological variables and no environmental variables.

Using only the 27 clinical variables resulted in Kappa coef-ficients with higher values and located completely inside the interval of substantial agreement, with the prioritized variables being: respiratory disorders, convulsion, shock, petechiae, coma, icterus, and diarrhea (**Table 2**).

The machine learning algorithms produced six rules (**Table 3**) that allow deducing that the evolution of a patient's case will be death.

Of the 104 cases confirmed as SF, 103 were from 25 munici-palities of RJ and one from the municipality of Guarulhos, São Paulo-SP. Ninety eight of these confirmed cases were found to be for patients who reside in 15 municipalities of RJ and 1 munici-pality (Tombos) of Minas Gerais (MG) (**Figure 4**).

DISCUSSION

This study was not able to make a diagnostic classification of suspected cases of SF through clinical signs and symptoms using
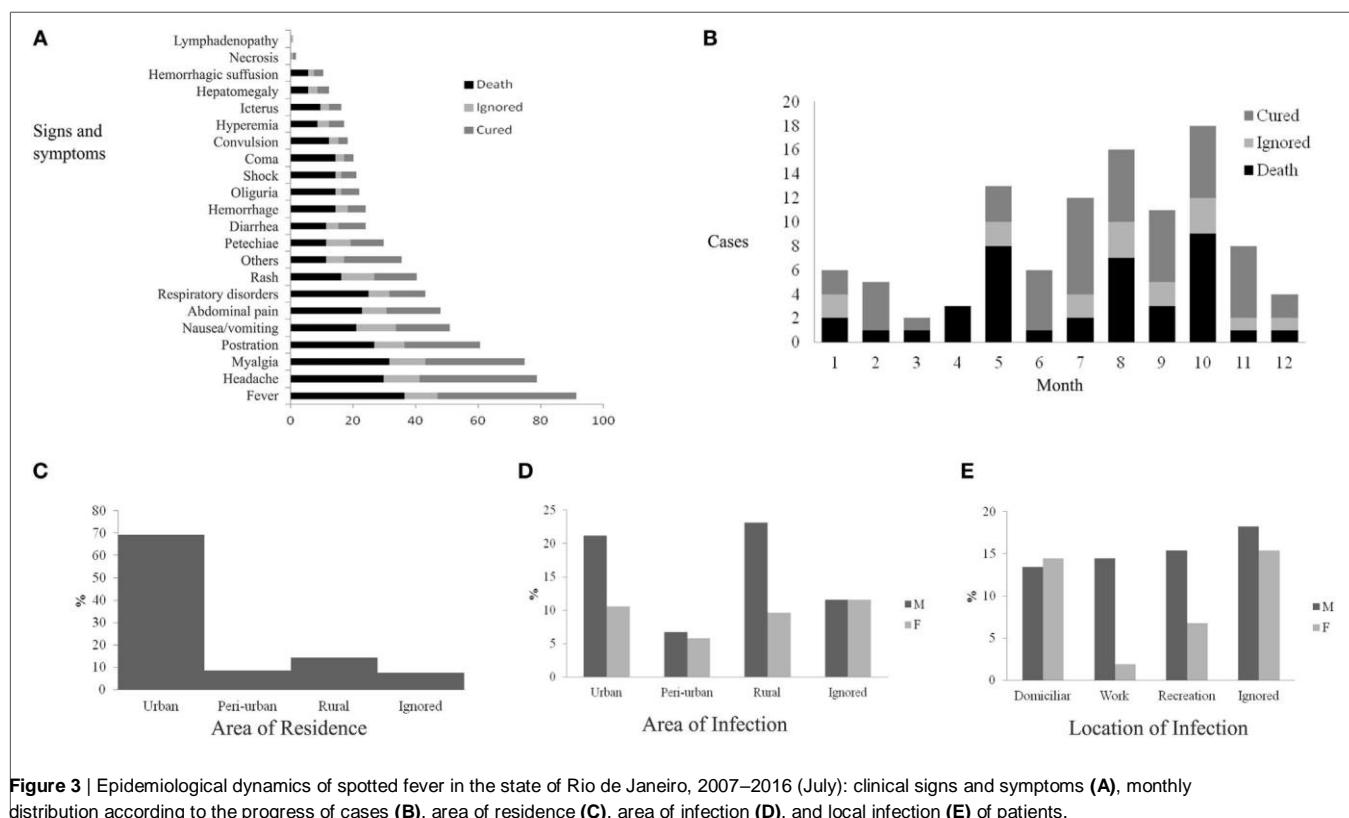
Figure 3 | Epidemiological dynamics of spotted fever in the state of Rio de Janeiro, 2007–2016 (July): clinical signs and symptoms **(A)**, monthly distribution according to the progress of cases **(B)**, area of residence **(C)**, area of infection **(D)**, and local infection **(E)** of patients.

Table 1 | Diagnosis classification using bayesian probabilistic classification neural network in the state of Rio de Janeiro.

Diagnosis	Cases	Correct instances
Cellulitis	2	0(0.0%)
Dengue	14	4(28.6%)
Encephalitis	1	1(100.0%)
Hapatitis A	2	0(0.0%)
Meningitis	3	0(0.0%)
Leptospirosis	7	2(28.6%)
Other disease	31	11(35.5%)
Spotted fever	38	21(55.3%)
Tick bite allergy	2	0(0.0%)
Virosis	2	0(0.0%)
Total	102	39(38.2%)

Table 2 | Classification of cases of spotted fever in the state of Rio de Janeiro as death or recovery using epidemiological variables and prioritized clinical variables.

Algorithm	Kappa	Correct instances (%)	Folds	Selected tree decision variables
Best First decision tree	0.2935	67.1	19	Contact with tick, cattles, woods-forest-river-waterfalls, other
J48	0.3648	70.5	22	Contact with woods-forest-river-waterfalls, other
Reduced-error pruning tree	0.3159	68.2	12	Contact with woods-forest-river-waterfalls, other
Best first decision tree	0.68	84.1	28	Respiratory disorders, convulsion
J48	0.62	80.9	8	Respiratory disorders, convulsion
Reduced-error pruning tree	0.62	80.7	4	Coma, convulsion, icterus, respiratory disorders, diarrhea

Table 3 | Prediction rules obtained by machine learning for death from spotted fever (SF) in the state of Rio de Janeiro.

Rule		Reliability (%)	Support (%)
R1	Respiratory disorders → death	63.2	61.5
R2	¬ Respiratory disorders ∧ convulsion → death	85.7	15.4
R3	Coma → death	100.0	30.8
R4	¬ Coma ∧ icterus ∧ respiratory disorders → death	100.0	10.3
R5	¬ Coma ∧ ¬ icterus ∧ convulsion → death	75.0	7.7
R6	¬ Coma ∧ ¬ icterus ∧ ¬ convulsion ∧ ¬ diarrhea ∧ respiratory disorders → death	60.0	7.7

Note that the possible consequences for patient disease are death or recovery, so the random probability of death is 50%. This means that any rule with confidence value higher than 50% is better than random choice. For each of these rules, we calculated the values of two metrics: support, which indicates the percentage of SF notification records in the sample space that endorse the rule; reliability, which indicates the percentage of SF notification records whose patients in fact died when presenting the clinical symptoms described in the rule.

techniques of neural networks. However, ML for knowledge representation provided good results. Rash and the presence of petechiae seem to be strong indicators of SF (5–7) and were present in 40.4% (42/104) and 29.8% (31/104) of the cases, respectively (**Figure 3**).

Although 71.0% (74/104) of the confirmed SF patients had contact with a tick and 69.2% (72/104) had performed some activity in nature, these were not factors unique to the disease. In fact, laboratory tests confirmed cases for dengue and lepto-spirosis, 53.3% (8/15) and 62.5% (5/8), respectively, in which subjects had also had contact with ticks. However, contact with ticks as a historical factor of suspected SF remains important (3, 25, 26), while contact with capybaras, present in 1.7% (2/104) of cases, is not a relevant factor in suspected SF in the state of RJ (27), as established in the surveillance protocols for Brazil (4, 5, 21, 22).

This study found that some changes need to be made to the SF notification report form (28). The "ignored" alternative, which appears in various fields/variables such as sex, area of residence, all clinical signs, and symptoms, among others, makes it difficult or even impossible to achieve a deeper understanding of the epidemiological dynamics of SF and evaluate the sensitivity of SINAN, as was the case in this study. Thus, we recommend binary responses for such fields (1 or 2).

Moreover, the separation of dogs and cats in Field 34, regard-ing Epidemiology group, seems to be important (28), since dogs have been shown to be an important amplifier for *R. rickettsii*, Brazil (29, 30), and they usually act as hosts for several species of ticks in endemic areas of SF (31–33).

Furthermore, we emphasize the importance of instructing qualified SS professionals on how to correctly complete the epidemiological investigation forms from SINAN. We noticed, for example, that the field responsible for recording the diagnosis (Field 51) was frequently filled improperly, which caused a 72.7% (647/870) drop in the original sample size of cases. In fact, this lack of information compromised the performance of the neural networks, resulting in a low overall percentage of correct clas-sification (45.6 and 37.3%; results not shown).

It is very important to mention that based on laboratory classi-fication criteria (4, 5, 21, 22), only 48.5% (33/68) of the cases were confirmed by indirect immunofluorescence assay (IFA), isolation, and histopathology; the remaining cases did not meet criteria for laboratory classification (see in detail in **Figure 2**). Moliterno (34) previously made this same observation for confirmed cases in RJ from 2004 to 2008.

According to the technical staff of SES-RJ (*personal com-munication*), there was a critical situation at SINAN regarding this issue; that is, cases appearing confirmed by isolation mostly corresponded to results of PCR techniques, because there was no option on the epidemiological form for PCR (28), and so the isolation option was selected instead.

As expected, the decision trees analysis reinforced the hypoth-esis that epidemiological variables are not predisposing factors for the clinical evolution of the patient, as some clinical signs and symptoms are (**Table 2**). These results suggest that two experts on SF would agree with each other with a high frequency in their prediction of the clinical evolution (death or recovery) of cases

Figure 4 | Flow of patients diagnosed with spotted fever (SF) in the state of Rio de Janeiro, 2007–2016 (July). **(A)** Area with SF patient flow, **(B)** flow between the municipality of residence and the municipality of notification, and **(C)** flow of patients from the municipality of infection to the municipality of their residence. ES, Espírito Santos; MG, Minas Gerais; SP, São Paulo.
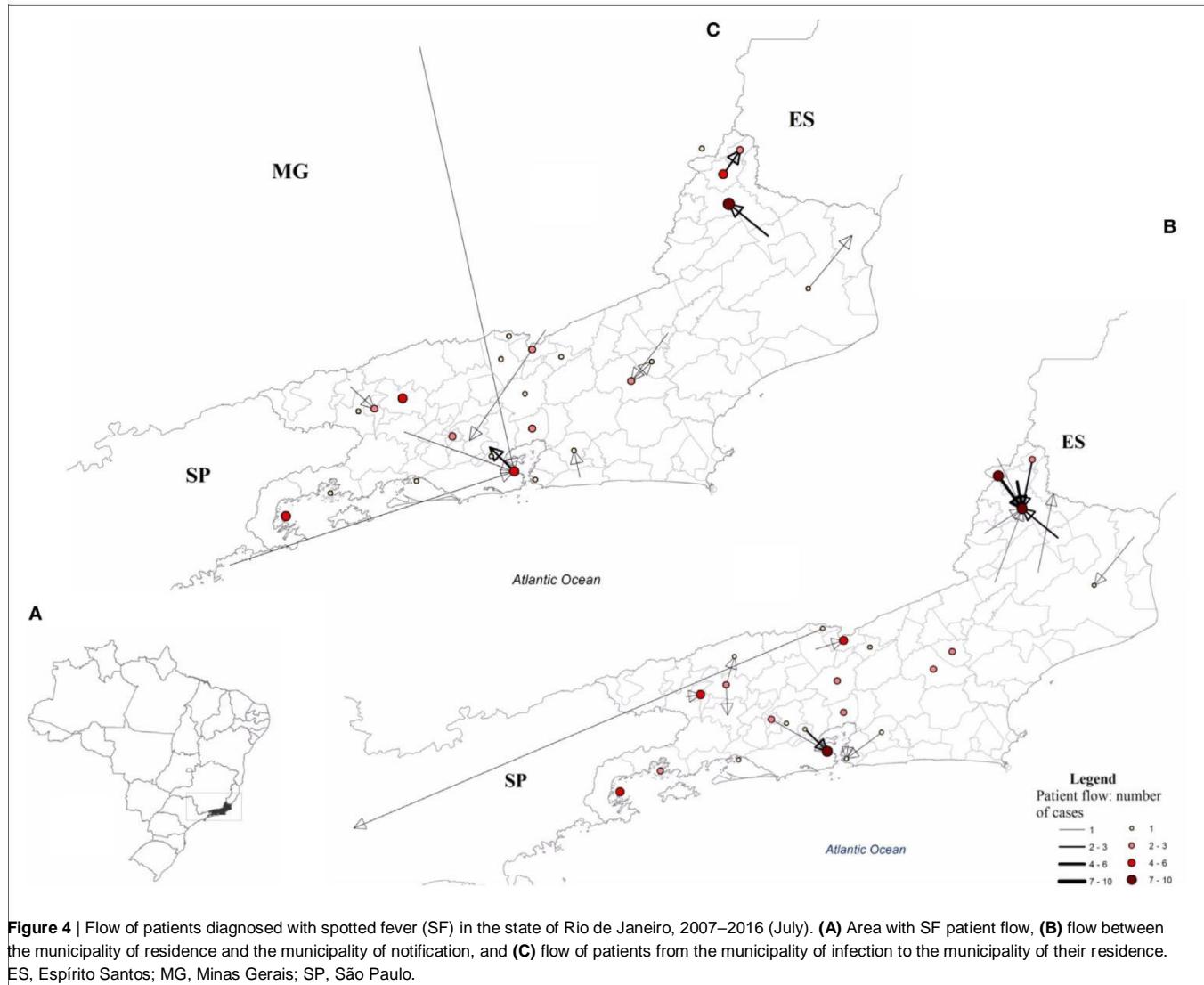
using the same clinical variables: respiratory disorders, convulsion, shock, petechiae, coma, icterus, and diarrhea. Some of these symptoms have also been associated with more severe clinical evolution and higher case-fatality by SF (3, 7, 25, 26).

In trying to prioritize symptoms, ML algorithms produced six rules (**Table 3**) that allow deducing that the evolution of a particular case will be death. Recall that any rule with a confidence value higher than 50% is better than a random choice, and thus increases the probability of predicting death. Rule R4, for example, is associated with 10.3% of the sample space with 100.0% confidence; in other words, the patient will die if he has coma or convulsion and also if he has respiratory disorders with or without icterus. This analysis produced intermediate Kappa coefficient values, located at the border between the classes seen as in moderate agreement and substantial agree-ment (35).

There is a dynamic flow of patients among RJ municipalities and bordering states (Espírito Santo, Minas Gerais, and São Paulo), which requires future work to integrate a more detailed

spatial component of the sites of infection for a greater understanding of the epidemiological dynamics of SF.

Overall, the findings here are of the utmost importance to SINAN and the SS for SF. They indicate that changes to the epidemiological form for SF are needed, that qualification of SS personnel should be improved, and that pilot studies should be established on sensitivity, focused in areas with a greater number of cases as well as epidemiological silent areas of the state of RJ.

Given the low quality of the SF case data in SINAN for the state of RJ, the artificial neural networks were not able to gener-ate robust predictive projections. Therefore, we recommend the selection of a set of municipalities with greater epidemiological burdens of SF in RJ for future prospective study applying these techniques.

Since some diagnostic categories are very rare, for example, encephalitis, and occur only a few times in the data set, it would be advisable to limit the exit space of the PNN to more frequent and related groups of pathologies, or to do so alone with SF and other pathologies. Comparative studies with other statistical tests

are needed, such as with Linear and Quadratic Discriminant Analysis.

AUTHOR CONTRIBUTIONS

DL—contributed to the concept and design; DL and FM—contributed to the design and application of M-L techniques and DL and CD with cartographic techniques; CD, PA, and MA with acquisition of the epidemiological information; FM, GG, and RB—contributed to concept and design of the research project, data acquisition, and interpretation of results. All authors contributed to critically revising the manuscript for important intellectual content and final approval of the version to be published. All authors are in agreement to be accountable for all aspects of the work and in ensuring that questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved.

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Capitulo 3. One World, One Health: A model for spotted fever

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Abstract

BACKGROUND Among the human diseases caused by etiological agents transmitted by ticks in Brazil, the most prevalent, and of great public health interest, is that caused by species of *Rickettsia*—Spotted Fever (SF). In the *Rickettsia* transmission cycle, different co-specific relationships between ticks and vertebrate hosts have been identified, and which are involved in various geographic, ecological and evolutionary processes. OBJECTIVE Apply the concept of "One World, One

Health" to achieve an integrated understanding of SF and determine vulnerability scenarios for human infection by pathogenic *Rickettsia* in the state of Rio de Janeiro (RJ). METHODS Multi-criteria decision analysis (PROMETHEE algorithm) and spatial statistics were performed on data encompassing epidemiology, health care, biotic determents and socioeconomic and demographic variables. The construction of multi-criteria descriptors used 33 indicators ordered in 12 sub criteria of 5 major categories (criteria): Public Health, Environment Health, Acarology, Veterinary Health and Microbiology. FINDINGS Spotted fever was found to occur non-randomly in RJ, exhibiting seasonality between March and April and between August and October. Determination of vulnerability was heterogeneous in the weight of contribution of indicators with the main contributors being those associated with the criteria Acarology (35.11%), Microbiology (33.25%) and Veterinary Medicine (23.96%). Spatially, and according to the Promethee algorithm, ticks of the species *A. sculptum* and dogs are essential determinants for the occurrence of human cases of SF in RJ. For defining indicators of SF and planning health actions, the "One World, One Health" concept proved to be applicable at three levels: **i.** the individual or probable areas of infection; **ii**. the population or municipality; and **iii.** the ecosystem or state. CONCLUSIONS The present work used the Promethee algorithm to transform qualitative perceptions to quantitative values for the different dangers and threats that make up socio-environmental vulnerability to human SF infection and defined the spatial dependence for infection with SF. In addition to being a model study for cases where weights of the contributions of each element of

the transmission chain are flexible according to the realities of the endemic areas, this study also demonstrates applicability from a national to a local (domicile) scale.

Keywords

One World, One Health, Tick-borne, Vulnerability, Spotted fever

Introduction

A total of 1415 species of infectious agents have been associated with human diseases throghout the world. Of these, about 60% (868) are considered zoonoses (121), representing 75% of emerging diseases and 80% of agents with bioterrorist potential (http://www.oie.int/en/for-the-media/onehealth/). These high prevalences have led to new considerations regarding understanding pathogens that have negative impacts on public and veterinary health (Fonte).

"One World, One Health", is a holistic and interdisciplinary vision that integrates ecosystem or environmental health, animal health, public health and food safety at local, country and global scales (122–126).

The concept, which has many historical antecedents, was presented in 2004 by the Wildlife Conservation Society (127). It is now widely accepted, incorporated and disseminated by the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO). The first joint efforts of this vision gave rise to the Global Early

45

Warning System for Major Animal Diseases Including Zoonosis – GLEWS (http://www.glews.net/).

Several interdisciplinary, cooperative and holistic studies have been developed towards understanding and combating a wide variety of pathogenic diseases (122,124–126,128–135).

In the present work, we consider the concept "*One World, One Health*" *sensu lato*; that is, as synonymous with "One Medicine", "One Health", Universal Health and Vulnerability, and Disease Ecology and Eco-epidemiology (122,124,125,132–134) for the prevention, surveillance and control of infectious diseases of zoonotic origin.

Among zoonoses, rickettsioses are produced by Gram-negative proteobacteria of the genus *Rickettsia* that are transmitted mainly by ticks to mammalian hosts and, accidentally, to man (16,136–138).

In Brazil, among the diseases caused by etiological agents transmitted by ticks, the most prevalent and of most interest to public health are Brazilian spotted fever (FMB) produced by *Rickettsia rickettsii*, and caused by other species of *Rickettsia* that are considered as emerging (species of the Spotted-Fever Group of *Rickettsia*-SFGR) such as *Rickettsia* strain Atlantic rainforest (24–26). However, for the purposes of the present study all these types of spotted fever will be treated collectively as Spotted Fever (SF).

In the transmission cycle of *Rickettsia*, different co-specific relationships between ticks and vertebrate hosts have been identified that involve geographical, ecological and evolutionary processes (42,64,70,91).Recent studies (Katz et al.,

2009; Brasil, 2014; de Oliveira et al., 2016; Montenegro et al., 2017) indicate that the dynamics of SF in the endemic states of Brazil is of a seasonal nature, with highest incidence rates in the second part of the year (June to October). This seasonality has been associated with the population dynamics of species of ticks of the genus *Amblyomma*, but particularly high densities of larvae and nymphs of *Amblyomma sculptum* (24,43–47). This tick is considered the main vector of *R. rickettsii* among the human population of Brazil (Brasil, 2009, 2014; Szabó et al., 2013; Montenegro et al., 2017).

However, there are a number of species of ticks that could be considered reservoirs and vectors of pathogenic *Rickettsia*, and some mammalian species that could function as amplifiers and reservoirs of these bioagents. These elements are potentially responsible for the occurrence and maintenance of epizootic cycles and the emergence and/or re-emergence of epidemic outbreaks of SF in Brazil. Although they are main determinants of bacterial transmission cycles, they are not significant hazards due to the establishment of risk and vulnerability areas, and surveillance programs, as well as efforts to control human outbreaks, as demonstrated by the findings of a number of studies (58,59,86,89–91).

An integrated approach is a promising strategy for understanding the dynamics of SF, and determining vulnerability scenarios for infection of humans by pathogenic *Rickettsia* because it would result in the establishment of SF surveillance and environmental control programs. This is precisely what we aim to achieve in the present work by employing the concept pf "*One World, One Health*". With the

establishment of a clear vulnerability scenario, clinically suspected cases in high vulnerability areas may be treated early (3,139).

Materials and methods

Study area

The state of Rio de Janeiro is located in the eastern portion of Brazil's Southeast Region, and occupies an area of 43,777.954 km^2 divided into 92 municipalities. It is the fourth smallest state (by area) in Brazil, yet has the highest population density (365.23 inhabitants/km^2) with an estimated population of 16,640,00 inhabitants. It is also the most urbanized state in the country, with 97% of the population living in cities (IBGE, 2016).

Epidemiological data and health care

The epidemiological data presented here was obtained from the Sistema de Informação de Agravos de Notificação - SINAN (Notifiable Diseases Information System) (140), and provided by the Secretaria de Estado de Saúde do Rio de Janeiro - SES/RJ (State Secretary Health of Rio de Janeiro). The data comprised cases confirmed by laboratory tests (PCR or Serology) and/or clinical and epidemiological nexus of SF between 2007 and 2016.

These data were made available with the protection of the identity of the patients; therefore, information such as names or addresses cannot be displayed at any time to comply with national ethical regulations (Brasil, 2012).

To assess access to health care, the following municipal indicators were used: number of health professionals (HP) and number of hospital beds (HB) per 100 thousand inhabitants. For these indicators we used the used the average of the information from the years 2010 and 2016 obtained from the Secretaria de Atenção em Saúde-SAS (Health Care Secretary) of the Ministério da Saúde do Brasil (Ministry of Health-MH) (http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/sas).

Biotic determinants

Information regarding potential vectors of SF and hosts of the tick was obtained from the database of the Laboratório de Referência Nacional em Vetores das Riquetsioses (LIRN- Labortory of the National Reference of Rickettsial Vectors), built from samples received and analyzed within the workflow of the Rede Nacional de Vigilância de Ambiental para Febre Maculosa e outras Rickettsioses (National Network for Environmental Monitoring for Spotted Fever and other Rickettsial Diseases) of MH from 2007 to 2016. The samples were collected in sampling units (specimens originating from the same host or environment), by cloth-dragging, visual searches on hosts and inspection of plant litter and abiotic surfaces by state and municipal health teams from Rio de Janeiro for environmental surveillance and investigation of cases of SF.

Ticks were identified using dichotomous keys, while species of *Rickettsia* infecting ticks were identified using PCR (See in detail in Montenegro et al. 2017).

To characterize conditions of climatic suitability throughout the study region, potential distribution models for *A. sculptum, A. aureolatum* and *A. ovale* (24), the main vectors of SF in Brazil, were produced using the Maxent algorithm version 3.2.1 (Phillips et al., 2005; Elith et al., 2011). For this we used seven climatic variables that we considered crucial to the biology of the species and which had already been evaluated for collinearity by principal component analysis (PCA) in Niche Software version 3.0: annual mean temperature, mean diurnal temperature range, maximum temperature in the warmest month, minimum temperature in the coldest month, annual precipitation, and precipitation in the wettest and driest months.

To generate the final models, we selected the logistic values for the final output of the 25 boostrap interactions. The mean number of repetitions was selected as the final model and converted into binary models to generate adequacy maps that were overlaid as a shape-file to subtract information about adequacy in relation to the municipality of occurrence of these vectors.

Socioeconomic and demographic data

Data for socioeconomic and demographic indicators were obtained from Brazilian census data acquired by the Instituto Brasileiro de Geografia e Estatística-IBGE (http://www.ibge.gov.br). The following indicators were used for the analysis: Municipal Human Development Index (MHDI), GINI Index (IG), Demographic Density (DD), Land Use Municipality (LUM), Percentage of Poverty in the Rural Area (PPRA), Degree of Urbanization (DU) and the Percentage of the Agricultural Contribution to the Municipal GDP (GDP-AM).

Analysis of data

The first phase of analysis evaluated whether human infections with SF-causing pathogens occur randomly with respect to time. Three statistical tests were used in statistical software (99), with at least two coincident tests with confidence levels of 95% determining randomness, or not, of human cases of SF.

In the second phase of analysis, two multi-criteria decision analyses (MDA) were performed, for vulnerability of human infection with pathogenic tick-borne *Rickettsia*. Multi-criteria decision analysis is a method that approaches a certain object from different characteristics that influence the occurrence of a given problem. The method has become increasingly used for understanding of events of interest in public health (Montenegro et al 2017b).

The following phases of MDA were defined: i) Structuring Phase, ii) Modeling Phase and iii) Evaluation Phase. The objective of the Structuring Phase is to understand the decision tree formed by the determinant variables of SF. The Modeling Phase mathematically expresses human vulnerability to SF and the Evaluation Phase presents the results of the model.

   i.   *MDA Structuring Phase*

To construct multi-criteria descriptors and decision-making processes, three criteria comprising 13 sub-criteria, which in turn include 34 indicators of similar nature and that are considered determinants of SF, were used (Box 1).

   ii.   *MDA* Modeling Phase

For modeling MDA, numerical values of criteria, sub-criteria and indicators were incorporated into the decision-making model by employing peer-to-peer comparison. This comparison method attributes a preferential value to two criteria, two sub-criteria or two indicators, which are graphically compared to one another using the D-sight program (Hayez et al. 2012). Theoretical weights, ranging from 1 to 100, for the components of the decision-making model were generated using the PROMETHHE method (preference ranking method for enrichment evaluation) through peer-to-peer comparison (Brans & Mareschal 1994).

It should be noted that the PROMETHEE method was developed in order to help the individual or collective decision maker. These methods serve to solve problems by selecting, or making arrangements of, possible alternatives (municipality, options, shares), subject to an assessment of various of criteria (variables, qualitative and quantitative indices, indicators, attributes, any criteria with numerical or nominal values), which may be in conflict with each other, and seek to simultaneously satisfy different solutions for decision making (Brans & Mareschal 1994, Mareschal & De Smet 2009).

The third phase of analysis involved using the scores resulting from the PROMETHEE model for spatial statistical analysis. Spatial dependence of vulnerability for each area assessed was analyzed using the Moran Local Index - LISA (Anselin 2010), with 9999 permutations, no spatial autocorrelation and statistical significance (LISAsig) considered to be greater than 0.05. All spatial statistical analyses were performed using the program TerraView (INPA 2010).

Finally, using data points from data on probable infection sites for confirmed SF cases (using addresses reported in the epidemiological form with Google Maps), we sought to focus on areas with the highest probability of human SF infection within the biomes, and the greatest spatial association with hosts and ectoparasites in RJ, using the program (141).

Box 1. Criteria, sub-criteria and indicators of multi-criteria decision analysis (MDA).

Criteria	Sub-criteria	Indicators	Sources
Public Health	Health care	HP	SAS
		HB	
		Ratio HP/HB	
	Social	MHDI	IBGE
		GI	
		PPRA	
Environmental Health	Land use	LUM	IBGE
		DU	
		GDP-AM	
	Demographic	DD	
	Vector fitness (Presence of vectors in the environment (not hosts))	Modeled presence	LIRN
		Real presence	LIRN
Acarology	Tick vector (proven *R. rickettsii* transmission)	*A-aureolatum*	LIRN
		A-sculptum	
		A-ovale	
		Rh-sanguineus	
	Tick carrier synanthropic (infected with *Rickettsia* but without proof of transmission to hosts and associated with anthropized environments)	*A-dubitatum*	
		D-nitens	
		Rh-microplus	
		A-longirostre	
	Tick wild (infected or not with *Rickettsia*)	*Ha-leporispalustris*	
		Ornithodoros sp.	
Veterinary Health	Reservoir animals (proven infection of tick)	*C-familiares*	
		H-hydrochaeris	
	Host animals (has not been shown to function as a reservoir)	*E-caballus*	LIRN
		B-taurus	
		F-catus	
		E-asinus	

| Microbiology | Parasitism in humans | Mula | |
		H-sapiens	
	Etiological agent	*R-rickettsii*	LIRN and Montenegro et al 2017.
		Spotted-Fever Group of *Rickettsia* – SFGR	
		Rickettsia	

Results

Human infections with pathogenic *Rickettsia* in RJ occur mainly between the months of March-April and August-October, with it being more pronounced in the latter (**Figure 1**).

Two of the three tests employed showed that the temporal dynamics of SF cases is not a sequence of temporally random events. The first test counted the number of times the data sequence was above and below the median, and determined that the sample size was too small to perform a valid test (it was inconclusive).

The second test counted the number of times a sequence went up or down (number of executions up and down = 46, expected number of runs = 77, Z statistic = 6.77, Pv = 1.3^{-11}) and the third was based on the sum of squares of the autocorrelation coefficients – *Box Pierce test* (based on the first 24 autocorrelation coefficients, Z = 37.3, Pv = 0.040).
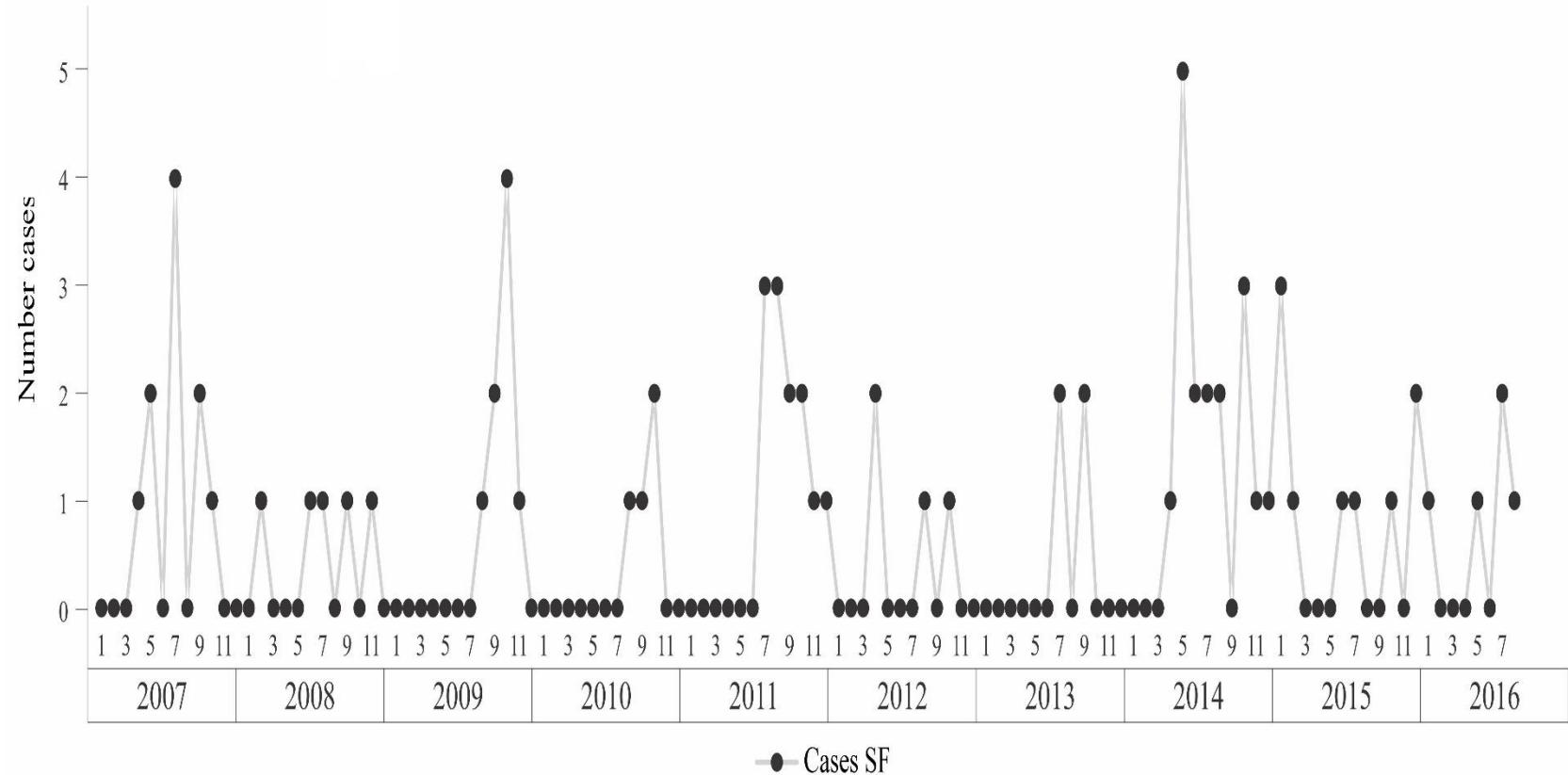
Figure 1. Temporal (monthly) dynamics of human infection with tick-borne pathogenic *Rickettsia* that causes spotted fever in the state of Rio de Janeiro.

Multi-criteria modeling found the vulnerability to tick-borne transmission of Spotted-Fever Group of *Rickettsia* in Rio de Janeiro to be heterogeneous with regard to the contribution of the weights of the two scenarios examined. The main sources of variation are in the contributions of the criteria Acarology (35.11%), Microbiology (33.25%) and Veterinary Medicine (23.96%) (**Table 1**). The five criterion are made up of 13 subcriterion, and 21, of the original 34 priority indicators and variables, contributed at least 2% to the two model of MDA.

. With the exception of Environmental Health, all criteria contributed equally to the determination of risk scenarios for SF; the greatest influence was by Acarology, with 27.3% of the total weight of the model. On the other hand, the vulnerability scenario was found to be more associated with Acarology and Micorbiology (**Table 1**). The five criteria are made up of 13 sub-criterion, and 21 of the original 34 priority indicators and variables; the indicators contributed at least 2% to the two model of MDA (**Table 1**).

According to the weight of each indicator, the largest (5%) absolute value generated by the Promethee algorithm for the occurrence of infection with SFGR are the criteria associated with the subcriteria Health care (rate HP/HB), Reservoir animals (*C. familiaris*), Human parasitism, Tick vector (*A. sculptum*) and Etiological agent (*R. rickettsia* and SFGR) (**Table 1**).

Table 1. Weights of the criteria used for determining vulnerability for tick-borne transmission of the Spotted-Fever Group of *Rickettsia* to humans.

Criterion	Sub-criterion	Indicators	Theorical Weight (%)	Absolute Weight (%) - Promethee
Public Health			**18.16**	
	Health care		75	
		Rate HP/HB	50	6.81
		HP	25	3.4
		HB	25	3.4
	Social		25	
		PPRA	33.33	1.51
		GI	33.33	1.51
		MHDI	33.33	1.51
Enviromental Health			**11.98**	
	Land use		29.7	
		GDP-AM	42.86	1.52
		DU	28.57	1.02
		LUM	28.57	1.02
	Demographic		16.34	
		DD	100	1.96
	Vector fitness		53.96	
		Real presence	66.67	4.31
		Modeled Presence	33.33	2.16
Veterinary Health			**23.96**	
	Reservoir animals		31.89	
		H-hydrochaeris	33.33	2.55
		C-familiares	66.67	5.09
	Host animals		22.11	
		Mula	9.59	0.51
		E-asinus	9.59	0.51
		F-catus	14.53	0.77
		B-taurus	30.46	1.61
		E-caballus	35.83	1.9
	Parasitism in humans		46	
		H-sapiens	100	11.02
Acarology			**35.11**	
	Tick vector		52.78	
		Rh-sanguineus	13.16	2.44
		A-aureolatum	25.23	4.68
		A-sculptum	42.43	7.86
		A-ovale	19.17	3.55
	Tick carrier			
		Rh-microplus	33.33	3.89
		D-nitens	33.33	3.89
		A-dubitatum	33.33	3.89
	Tick wild		13.96	
		Ornithodoros sp.	33.33	1.63
		Ha-leporispalustris	33.33	1.63
		A-longirostre	33.33	1.63
Microbiology			**33.25**	
	Etiological agent		100	
		Rickettsia	9.53	1.03
		SFGR	24.99	2.7
		R-rickettsii	65.48	7.07

The multi-criteria evaluation of the results of the PROMETHEE method found heterogeneous vulnerability scores for the 92 municipalities of the state of RJ. Based on the scores, 25% (23/92) of the territories are located in the highest level (4th quartile) of vulnerability for SF (**Figure 2a**).

The LISA technique identified 48.9% (45/92) of the municipalities as having high spatial dependence, with 19.6% (18/92) are in the category High (High - High) and 29.3% (27/92) in the category Low (Low – Low). Were found for the same categories of spatial dependence mentioned for vulnerability (**Figure 2b**). Statistically significant spatial correlation was found in 13.0% (12/92) of the municipalities (LISASig ≤5%) (**Figure 2c**).

The vulnerability model was sensitive to identifying areas with human SF infection (**Figure 2d**).
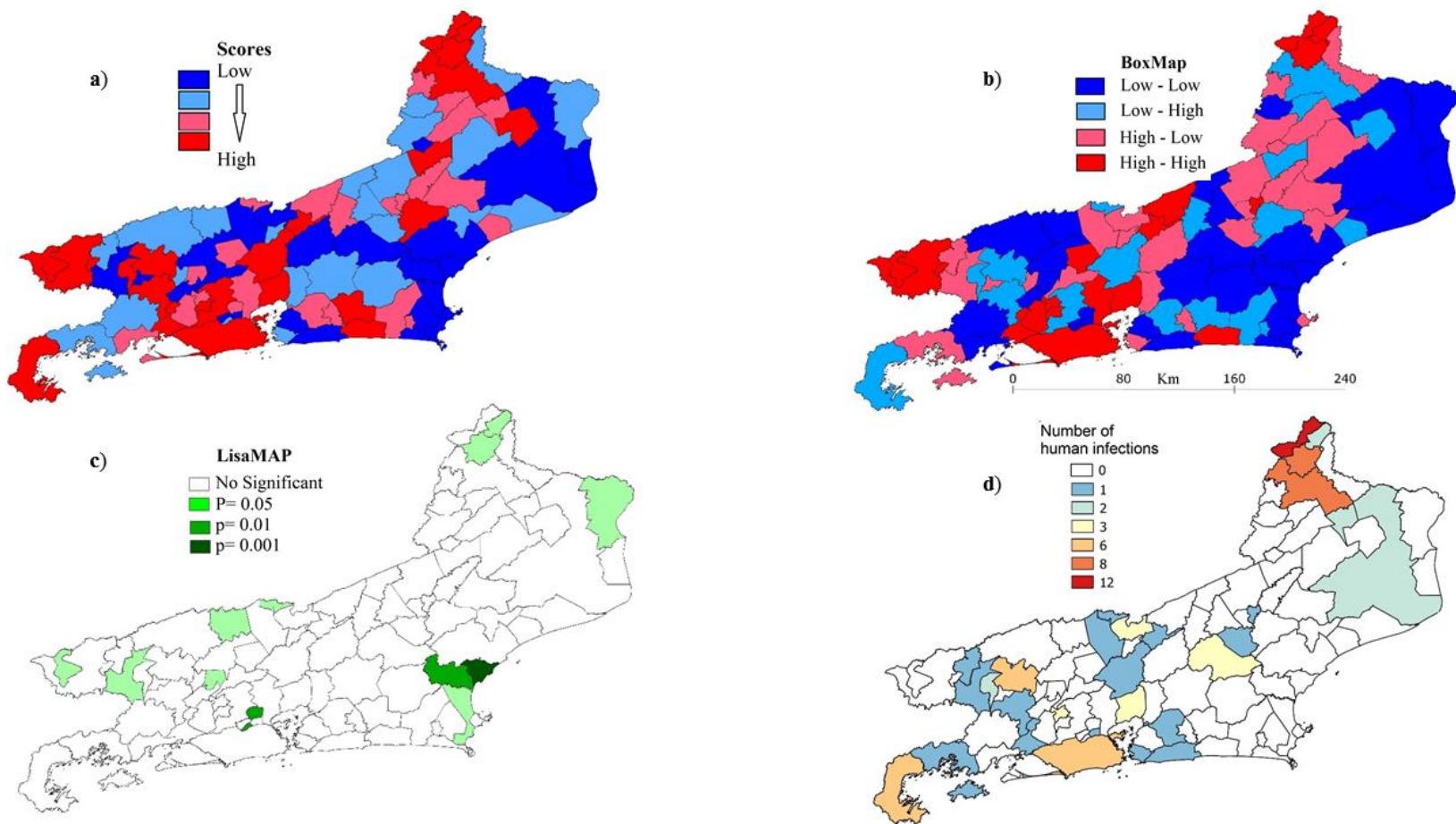
Figure 2. Spatial vulnerability for SF in the state of Rio de Janeiro. The letter **a** corresponds to the range of absolute values, **b** to spatial dependence, **c** to the statistical significance of spatial correlation and **d** to the number of cases with local infection.

Finally, it was possible to georeference 50% (39/78) of the confirmed cases of SF with probable areas of infection (PAIs) in RJ. The PAIs are located mainly in the areas where the Atlantic Forest biome and anthropic areas intersect (**Figure 3**). The ticks *A. sculptum* and *R. sanguineus* were the most frequent and abundant species in the PAIs. While dogs and the environment are the most significant host and site of infestation, respectively, with the most ticks occurring in places where humans acquire rickettsias.

The municipalities of Porciúncula, Natividade and Itaperuna are located in a hostpot for human acquisition of bacterias (**Figure 3**).
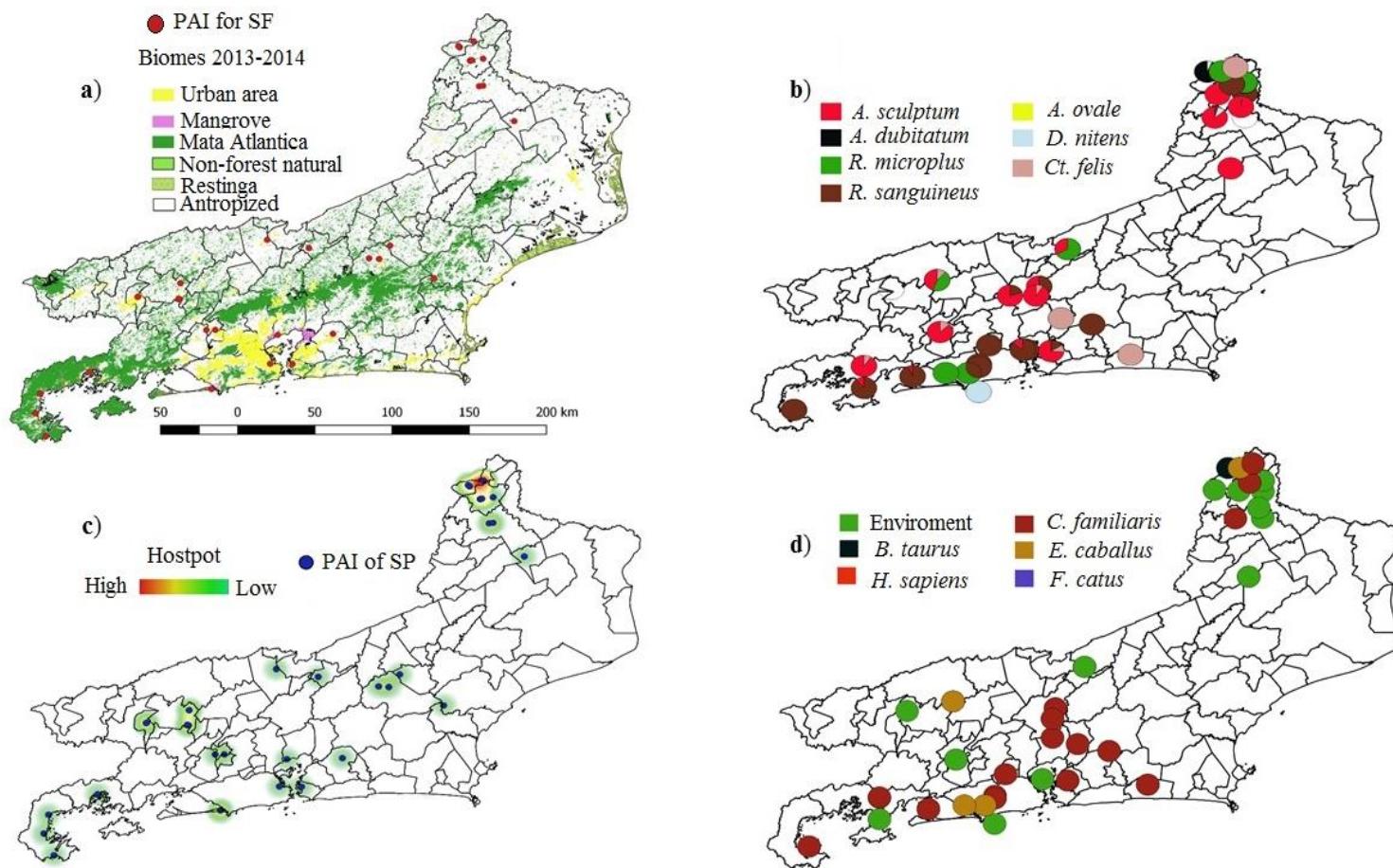
Figure 3. Distributions of biological and environmental determinants of probable areas of infection (PAIs) of pathogenic *Rickettsia* in the state of Rio de Janeiro, 2007-2016: **a**) PAIs relative to the biomes of RJ, **b**) tick species, **c**) hostpots for SF with 5Km radius and **d**) host and environment infested with ticks.

Discussion

In the state of RJ, human infections with pathogenic *Rickettsia* are an event of significant public health interest that do not happen randomly; there is seasonality with occurrence between March and April and between August to October, as in all endemic states of Brazil (3,11,26,42). This understanding requires that collective health actions be deployed before, during and after these periods, in order to combat the scenario of high mortality in RJ (42).

There exists a spatial association between increased frequency and abundance of the tick species *A. sculptum* and *R. sanguineus*, the presence of dogs and the environment (grass and vegetation) infested. At least spatially, and according to the Promethee algorithm, *A. sculptum* and dogs are essential determinants for the occurrence of human cases of SF in RJ. However, we emphasize the need for focused studies on the participation of *R. sanguineus* in outbreaks in RJ (42).

In the state of São Paulo, –Brazil, this specific tick of dogs was demonstrated to be involved in the rickettsial transmission cycle from the wild to the peridomestic environment. This tick functions as an amplifier and vector among hosts, thereby maintaining sources of infection for other species of vector ticks (*Amblyomma aureolatum*) in the peridomicile environment (142).

Recent studies of SF epidemiology in RJ have found that 69% (72/104) of SF cases confirmed in the last 10 years were in urban areas, and the rate of lethality for people infected in the peri-urban environment is greater (86.6%, 13/15). Thus, in

addition to solving this problem, it is also necessary determine the degree to which the dog tick participates in urban outbreaks and what strains of *Rickettsia* it amplifies.

The application of the concept of "*One World, One Health*" in the present study addresses three scales for defining indicators and health actin plans: **i.** the individual or PAIs; **ii.** the population or municipality; and **iii.** the ecosystem or state. The first level includes hotspots for human acquisition of the bacteria (**Figure 3c**). These same areas possess the greatest number of biological elements in the PAIs, and thus are the places where advertising posters should be placed announcing the risk of SF acquisition (3).

Note that Health Care represents a considerable weight in the categorization of vulnerability (13.61%), indicating that inter-sectoral municipal policies (second level) could have an impact on this indicator. Ensuring that the number of health care sites, the number of health professionals and, above all, the actions of epidemiological surveillance and health care, including treatment of suspected patients, are sufficient to reverse the lethality coefficients in the state, even without confirmatory evidence (3,139).

The third level serves to define and prioritize indicators in public health and environmental health, as well as interdisciplinary, to intervene and monitor in the state of RJ. The adequacy of resources and the development of pilot eco-epidemiological studies at a subregional level, following the patterns of vulnerability and spatial dependence (**Figure 2a** and **b**), are also recommended.

Despite the fact that climate data were used for modeling suitability for the presence of the principal vectors in Brazil, these variables (temperature, humidity,

rainfall, elevation, etc.) did not permit a local evaluation, as recommended (38,58–60,57,61–65). This limitation is associated with the low number of georeferenced cases (50% of the cases confirmed with PAIs were in RJ), and the lack of a necessary control of georeferenced unconfirmed cases of SF.

We urgently recommend that all cases (suspected and confirmed) be georeferenced. For this purpose we recommend using the free, internet-based GPS application Essentials, which also functions from satellites without the need of an internet connection (http://www. gpsessentials.com/).

Although this is not the first interdisciplinary effort to integrate different determinants for understanding SF (24,142), it is the first in Brazil to use multicriteria analysis with mathematical algorithms applied to that pathology.

The present work uses the Promethee algorithm to transform qualitative perceptions to quantitative values of the different dangers and threats that comprise the socio-environmental vulnerability of human infection, and defines the spatial dependence for infection with SF. Therefore, in addition to being a model study for cases where weights of the contributions of each element of the transmission chain are flexible according to the realities of the endemic areas, this study also demonstrates applicability from a national to local (domicile) scale.

Conflict of interest statement

The authors declare that we have prepared the manuscript in accordance with the standards of the journal, possess exclusive responsibility for the accuracy and correctness of the contents of the submitted article and declare that we have no conflicts of interest. The opinions expressed by authors contributing to this

journal do not necessarily reflect the opinions of the journal or the institutions with which the authors are affiliated.

Author contributions

DM - contributed to the concept, design and application of MDA techniques and spatial statistics; APC and MP – contributed to the design and application of MDA techniques and spatial statistics; -SVO made the climate suitability modeling for vector; SVO, GSG and RPB - contributed to research project design and concept, data gathering and interpretation of results. All authors contributed to critically revising for important intellectual content; final approval of the version to be published; and all are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Reference (Vide item 6).

65

5 CONCLUSÕES

1. No RJ circulam artrópodes infectados com *Rickettsia felis*, *Rickettsia bellii* e *Rickettsia rickettsii*, sendo modelados por seus hospedeiros específicos. Sugerindo que a associação entre cães, bovinos, cavalos, capivaras e seus principais ectoparasitos, *Rhipicephalus sanguineus* e *Ctenocephalides felis*; *Rhipicephalus microplus*; *Dermacentor nitens;* e *Amblyomma dubitatum*, respectivamente, têm papel fundamental na dinâmica da transmissão de *R. rickettsii* em ciclos enzoóticos e na manutenção de populacões de vetores infectados, que proporcionam a existência de áreas endêmicas com a oportunidade de surgirem surtos epidêmicos de FM.

2. O parasitismo em humanos só foi confirmado por *Amblyomma sculptum* infectado com *Rickettsia rickettsii*, o que reforça a importância dessa espécie como vetor do patógeno no Brasil.

3. O contato com carrapato, como antecedente para captar casos mórbidos por *Rickettsia*, continua sendo importante, e definitivamente, o contacto com capivaras, não é um fator relevante na suspeita de FM no RJ, como está estabelecido nos protocolos de vigilância do Brasil.

4. A dinâmica epidemiológica da FM no RJ está relacionada à percepção da doença no contexto de políticas de saúde em diferentes períodos de tempo, o que parece ter influenciado a sensibilidade do sistema de saúde na detecção de casos.

5. No RJ, rotinereimente, a primeira suspeição clínica é de leptospirose ou dengue, e quando o caso progride com complicações sistêmicas, até a morte, se suspeita de FM, o que direitamente incide na alta taxa de letalidade.

6. Confirmamos que não é possivel fazer uma classificação diagnóstica dos casos suspeitos de FM através dos sinais e sintomas clínicos.

7. São necessárias algumas mudanças na ficha epidemiológica pela FM. Os dados analisados dificultaram a compreensão da dinâmica epidemiológica e a avaliação da sensibilidade do Sistema de Vigilância. Recomendamos deixar respostas binárias excludentes nas variáveis do formulário (1 ou 2).

8. Ressaltamos a importância de qualificar os profissionais responsáveis pelo preenchimento das fichas de investigação epidemiológica do SINAN.

9. É muito importante mencionar que baseado nos critérios de classificação laboratorial dos casos, no Brasil, cerca de 50% dos casos não cumpriram esses critérios. Recomenda-se que os técnicos da vigilância epidemiológica façam uma análise detalhada dos critérios utilizados para confirmação ou descarte dos demais casos que aparecem confirmados por laboratório (35 em total).

10. Espacialmente se evidenciou a dinâmica de fluxo de pacientes entre municípios do RJ e entre estados fronteriços (Espírito Santos-ES, Minas Gerais-MG e São Paulo-SP). Deve ser também retirado um dos casos confirmados no RJ porque reside em MG.

11. No RJ, as infecções humanas com *Rickettsia* patogênica não acontece aleatoriamente; há sazonalidade com ocorrência entre março e abril e entre agosto e outubro, como em todos os estados endêmicos do Brasil. Este

entendimento exige que as ações coletivas de saúde sejam implantadas antes, durante e após esses períodos, a fim de combater o cenário de alta mortalidade em RJ.

12. Pelo menos espacialmente, e de acordo com o algorítmo Promethee, *A. sculptum* e cães são determinantes essenciais para a ocorrência de casos humanos de FM em RJ. No entanto, enfatizamos a necessidade de estudos focados sobre a participação do carrapato *R. sanguineus* nos surtos no RJ.

13. A vulnerabilidade espacial na infecção humana com rickettsias patogênicas transmitidas pelos carrapatos pode ser analizada em três níveis: **i.** O individual ou LPI; **ii.** A população ou município; e **iii.** O ecossistema ou estado.

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