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HELEN ALINE MELO

Análise geoestatística de áreas de risco de leishmaniose tegumentar no estado do Paraná

Maringá
2017

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para a obtenção do título de Doutor em Ciências da Saúde.
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meus pais, Ana Maria e José Guido

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“Comece fazendo o que é necessário,
depois o que é possível, e de repente
você estará fazendo o impossível.”
(FRANCISCO DE ASSIS)

Análise geoestatística de áreas de risco de leishmaniose tegumentar no estado do Paraná

RESUMO

A distribuição geográfica da leishmaniose tegumentar (LT) em todos os estados mostram a importância desta doença no Brasil. Na região sul, o maior número de casos de LT ocorre no estado do Paraná. O estudo da distribuição espacial de LT neste estado, utilizando a análise geoestatística, facilita a determinação de áreas de maior risco e a compreensão da influência das ações do homem para a ocorrência da doença nestas áreas. Este estudo é composto de dois trabalhos. No primeiro foram utilizados o índice global de Moran, índice local de associação espacial (LISA) e confecção de mapas por categorias de associação direta (high-high e low-low) e associação negativa (high-low e low-high), com dados do período de 2001 a 2015 do Sistema de Informação de Agravos de Notificação (SINAN) de casos autóctones de LT no estado do Paraná. Neste estado, de 2001 a 2015, ocorreram 4.557 casos, com média anual de 303,8 (±135,2), o coeficiente de detecção foi 2,91 e a densidade de casos por km^2 foi 0,023, em 268 municípios. Os municípios com maior coeficiente de detecção foram Jussara (237,47), Adrianópolis (165,03), Cerro Azul (108,29), Ivatuba (108,11) e Japurá (89,35). O índice global de Moran para o coeficiente de detecção de casos autóctones de LT para cada um dos anos de 2001 a 2015 mostra estatisticamente a existência de autocorrelação espacial ($p<0,05$), exceto no ano de 2001 ($I=-0,456$ e $p=0,676$). Os mapas de distribuição de LT no estado do Paraná evidenciam a existência de *clusters* em áreas de produção desta doença nos pólos Ivaí-Pirapó, Tibagi, Cinzas-Laranjinha e Ribeira. No segundo trabalho o objetivo foi avaliar a influência da vegetação remanescente do estado do Paraná e a ocorrência de casos de LT. Foram utilizados dados de casos autóctones de LT, de 2012 e 2013, segundo o SINAN, e dados de vegetação remanescente da Fundação SOS Mata Atlântica e Instituto Nacional de Pesquisas Espaciais (INPE). Foi usada a modelagem SAR (simultaneous autoregressive model), tendo como variável independente o coeficiente de detecção de casos autóctones de LT e as variáveis dependentes, no caso, a vegetação natural (km^2), o percentual de vegetação natural, a altitude, o total de casos e a densidade de casos por km^2. Em 2012 e 2013 ocorreram 513 casos de LT, em 85 (21,30%) de 399 municípios, no estado do Paraná. Os municípios com maiores coeficientes de detectação nos anos de 2012 e 2013 foram: Jussara (191,83), Japurá (113,06), São Tomé (109,19), Tomazina (103,48), Adrianópolis (102,39) e São Jerônimo da Serra (78,73). Os cálculos de regressão dos dados revelam significância somente para a densidade de

casos (Z=22,1359, p=<2e-16), sugerindo que há risco da ocorrência de LT em áreas em que a vegetação remanescente de floresta estacional semidecidual está mediamente ou muito alterada nos pólos de produção Ivaí-Pirapó, Tibagi e Cinzas-Laranjinha. O uso de georreferenciamento espacial é útil para a determinação de áreas de risco de LT, porém não se deve subestimar o conhecimento das condições ambientais locais que exercem profunda influência na epidemiologia desta doença.
Palavras-chave: Leishmaniose Tegumentar; Epidemiologia; Zoonose; Análise Espacial

Geostatistical analysis of areas of risk of cutaneous leishmaniasis in the state of Paraná

ABSTRACT

The geographic distribution of cutaneous leishmaniasis (CL) has demonstrate great importance in all states of Brazil. The state of Paraná is responsabible for the highest number of CL cases in southern region. The use of the geostatical analysis for understand the spatial distribution of CL, facilitates to determine the high-risk areas and understand the influence of man's actions for occurrence of the disease in these areas. This study is composed of two works. At first, we used the global Moran index local index of spatial association (LISA) and making categories maps of direct association (high-high and low-low) and negative association (high-low and low-high), with data for the period 2001-2015 the Sistema de Informação de Agravos de Notificação (Information System for Notifiable Diseases - SINAN) of autochnous cases of CL in Paraná. In this state, from 2001 to 2015, there were 4,557 cases with average of 303.8 (± 135.2), detection rate was 2.91 and spatial density per km^2 was 0.023 at 268 municipalities. The municipalities with the highest detection coefficient were Jussara (237.47), Adrianópolis (165.03), Cerro Azul (108.29), Ivatuba (108.11) and Japura (89.35). Moran global index for the CL autochthonous case detection coefficient for each of the years 2001 to 2015 shows statistically the existence of spatial autocorrelation ($p < 0.05$), except for the year 2001 ($I = -0.456$ e $= 0.766$). CL distribution maps in the state of Paraná show the existence of *clusters* in areas of production of this disease in the Ivaí-Pirapó, Tibagi, Cinzas-Laranjinha and Ribeira hubs. Second study was objective to evaluate the influence of remaining vegetation of the state of Paraná and occurrence of CL cases. Data from autochthonous cases of CL, 2012 and 2013, according to SINAN, and data on remaining vegetation of the Fundação SOS Mata Atlântica (SOS Mata Atlântica Foundation) and the Instituto Nacionais de Pesquisas Espaciais (INPE; National Institute of Spatial Research) were used. The simultaneous autoregressive model (SAR) model was used, having as independent variable the coefficient of detection of CL autochthonous cases and the dependent variables, in this case, natural vegetation (km^2), percentage of natural vegetation, altitude, total of cases and spatial density per km^2. In 2012 and 2013, 513 cases of CL occurred in 85 (21.30%) of 399 municipalities in Paraná state. Municipalities with the highest detection coefficients in 2012 and 2013 were: Jussara (191.83), Japurá (113.06), São Tomé (109.19), Tomazina (103.48), Adrianópolis (102.39) and São Jerônimo da Serra (78.73). Regression calculations of data reveal significance only for the spatial density ($Z = 22{,}1359$, $p = <2e\text{-}16$),

suggesting that there is a risk of CL occurrence in areas where the remaining vegetation of semideciduous seasonal forest is moderately or much altered in the production centers Ivaí-Pirapó, Tibagi and Cinzas-Laranjinha. The use of spatial georeferencing is useful to determination of areas at risk of CL, but one should not underestimate the knowledge of local environmental conditions that exert a profound influence on the epidemiology of this disease.
Keywords: Cutaneous Leishmaniasis; Epidemiology; Zoonosis; Spatial Analysis.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

Tese elaborada e formatada conforme as normas da ABNT (Capítulo I) e das publicações científicas (Capítulo II): PlosOne (artigo 1) disponível em:
< https://goo.gl/vqPAhY > e Memórias do Instituto Oswaldo Cruz (artigo 2) disponível em:
< https://goo.gl/YQJj3Q >.

SUMÁRIO

CAPITULO I

INTRODUÇÃO

A leishmaniose tem três formas clínicas distintas: leishmaniose visceral (LV), que pode ser fatal se não tratada; leishmaniose tegumentar (LT), que é a forma mais comum e a leishmaniose mucocutânea (LM), que tem como característica a desfiguraração do paciente (OMS, 2017). A leishmaniose está amplamente distribuida no mundo, onde aproximadamente 350 milhões de pessoas vivem em áreas risco de infecção por *Leishmania* (DESJEUX, 2004).

O Brasil tem destaque na ocorrência de casos de leishmaniose, principalmente em relação à LT, pois de 1993 a 2012, a média anual casos autóctones notificados foi 26.965, com coeficiente de detecção (casos autóctones/100.000 habitantes) de 15,7 (BRASIL, 2016). A LT é endêmica no Brasil, com aumento de casos ao longo do período referido, destacando-se os anos de 1994 e 1995, com coeficientes de detecção de 22,83 e 22,94, respectivamente (BRASIL, 2016). Os dados mostram a expansão geográfica desta dermatose no Brasil, pois na década de 80 do século passado havia registro de casos em 19 estados, enquanto em 2003 ocorreram casos em todos os estados (BRASIL, 2016).

O estado do Paraná responde pelo maior número de casos de LT na região sul do país, no período de 1980 e 2014, atingindo 15.456 casos (BRASIL, 2006; 2016). Historicamente os casos de LT são constatados no norte, oeste e sudeste do Paraná, com casos relatados no início do século XX, ao lado da expansão da monocultura cafeeira (LUZ et al., 2000). As epidemias nos anos de 1930 a 1950 surgiram como consequência das ações humanas no processo de colonização do interior deste estado (LUZ et al., 2000). Cabe lembrar que as ações para o controle e erradicação da malária na década de 50, com o uso de inseticidas clorados, refletiram indiretamente no controle da LT no Paraná, diminuindo drasticamente o número de casos (LUZ et al., 2000). Porém, o registro de casos da doença a partir de 1980 mostra o retorno da enfermidade, com característica endêmica (SILVEIRA et al., 1996; SILVEIRA et al., 1999; LIMA et al., 2002).

A ineficiência dos serviços de saúde pública e o despreparo de profissionais têm dificultado as ações de controle de vetores, que é fundamental para o controle da LT (SEN GUPTA, 1975; ALEXANDER, MAROLI, 2003). O que requer a sensibilização de populações que vivem em áreas de risco para usarem medidas baratas e práticas que venham a ser incorporadas na sua rotina diária para o controle da doença (ALENCAR, 1983; TOWNSON et al., 2005). Este foco tem sido o objetivo de trabalhos efetuados no estado do Paraná (TEODORO et al., 1993; 2001; 2003, 2006; LUZ et al., 2000; MEMBRIVE et al., 2004;

MASSAFERA et al., 2005; CELLA et al., 2011; CRUZ et al., 2013; REINHOLD-CASTRO et al., 2013).

O conhecimento da distribuição geográfica da LT permite formular hipóteses sobre as variáveis geográficas e ambientais que influenciam a produção da doença, assim como no planejamento e direcionamento de políticas públicas de saúde de ações mais adequadas para o controle, especialmente de vetores (KING et al., 2004). O modelo de colonização das mesorregiões norte central, centro ocidental e noroeste do estado do Paraná, como fator de risco para LT, destaca que as condições necessárias à produção dessa doença foram criadas possivelmente no processo de ocupação e organização do espaço rural das mesorregiões referidas e, posteriormente, com a mudança do cultivo de café para outras culturas, provocada pela crise da monocultura cafeeira (MONTEIRO et al., 2008). A densidade de casos de LT mostra a ocorrência de dois circuitos da produção da doença no estado do Paraná, o circuito Paraná-Paranapanema, constituido dos pólos Cinzas-Laranjinha, Tibagi, Ivaí-Pirapó, Piquiri e Baixo Iguaçu, e o circuito Ribeira, representado pelo o pólo Alto Ribeira (MONTEIRO et al., 2009). Anteriormente, Lima et al. (2002) alertaram sobre a possibilidade de ocorrência de LT em outras areás no estado do Paraná, com características ambientais semelhantes às dos municipios de Cianorte, Japúrá, Jussara e São Tomé.

A compreensão da distribuição de agravos a saúde é importante para estabelecer ações de vigilância, prevenção e controle (MEDRONHO et al., 2003; EISEN, EISEN, 2008; MAGALHÃES, 2012). A análise estatística de dados do geoprocessamento facilita compreensão da distribuição de agravos e possibilita a confecção de mapas de risco de agravos à saúde (MAGALHÃES, 2012).

AGENTE ETIOLÓGICO

A leishmaniose é causada por protozoários parasitos intracelulares do gênero *Leishmania*, transmitidos pela picada de insetos denominados flebotomíneos. Os parasitos apresentam-se em duas formas principais: uma flagelada ou promastigota, encontrada no tubo digestório do inseto vetor, e outra aflagelada ou amastigota, observada nos tecidos dos hospedeiros vertebrados (BRASIL, 2007, 2016).

No Brasil, foram identificadas sete espécies do gênero *Leishmania*, sendo seis do subgênero *Viannia* e uma do subgênero *Leishmania*. As três principais espécies que causam leishmaniose nesse país são: *Leishmania (Leishmania) amazonensis* (Lainson & Shaw, 1972); *Leishmania (Viannia) guyanensis* (Floch, 1954): *Leishmania (Viannia) braziliensis* (Vianna, 1911) (BRASIL, 2016). *Leishmania amazonensis* causa lesões cutâneas e eventualmente difusas (anérgicas) e ocorre desde a América Central até o norte, nordeste e sudeste da América do Sul; *Leishmania guyanensis* causa predominantemente lesões cutâneas e ocorre na parte da América do Sul, restrita à bacia amazônica; *Leishmania braziliensis* causa lesões cutâneas e mucosas com ampla distribuição geográfica da América Central ao norte da Argentina (BRASIL, 2016).

VETORES

A relação vetor-hospedeiro tem interesse epidemiológico, pois o conhecimento do hábito alimentar e dos animais mamíferos como fontes sanguíneas esclarece a participação de ambos no ciclo de *Leishmania*. Miranda et al. (1998) afirmam a fonte sanguínea, juntamente com os parâmetros ecológicos, determinam a distribuição e a possível ocorrência da doença. A atuação de um flebotomíneo como vetor depende do sucesso do parasito ao multiplicar-se no interior do seu tubo digestório, o que por sua vez está na dependência da habilidade do parasito em superar as barreiras, tais como a resistência à ação de enzimas digestivas, capacidade de atravessar a membrana peritrófica e aderir às células epiteliais no tubo digestório deste inseto (KILLICK-KENDRICK, 1990; PAUL READY, 2013).

Na América existem cerca de 500 espécies de flebotomíneos conhecidos, entretanto apenas 30 espécies são vetores de leishmaniose. Aa fêmeas realizam a hematofagia para o desenvolvimento dos ovos (Figura 1). Durante esse procedimento pode ocorrer o consumo de sangue infectado por formas amastigotas de *Leishmania* sp. que após o período de quatro a 25 dias se modifica para as formas promastigotas que são inoculadas juntamente com a saliva durante o repasto sanguíneo no hospedeiro vertebrado (OMS, 2017). As espécies de flebotomíneos *Nyssomyia neivai* (Pinto), *Nyssomyia whitmani* (Antunes & Coutinho) e

Migonemyia migonei (França) foram identificadas como vetores de *Leishmania* (LUZ et al. 2000; OLIVEIRA et al. 2011; NEITZKE-ABREU et al. 2014), com ampla distribuição no estado do Paraná (SILVA et al. 2008).

Figura 1. Fêmea de flebotomíneo ingurgitada durante o processo de hematofagia. Fonte: OMS (2017)

Os estudos sobre a fauna e o comportamento de flebotomíneos, no Estado do Paraná, têm contribuido para esclarecer a epidemiologia da LT e as ações de controle desses insetos (TEODORO et al., 1993; 2001; 2003, 2006; LUZ et al., 2000; MEMBRIVE et al., 2004; MASSAFERA et al., 2005; CELLA et al., 2011; CRUZ et al., 2013; REINHOLD-CASTRO et al., 2013).

HOSPEDEIROS VERTEBRADOS E RESERVATÓRIOS

Na América mais de 40 espécies de mamíferos silvestres de ordens as mais distintas são conhecidas como reservatórios de *Leishmania* spp. (GRIMALDI, TESH, 1993). No Brasil, a infecção natural por *Leishmania braziliensis* foi detectada em roedores dos gêneros *Oryzomys*, *Akodon*, *Holochilus*, *Proechimys*, *Rattus*, *Rhipidomys*, *Bolomys*, *Nectomys* e marsupiais *Didelphis* (FORATTINI et al., 1972; LAINSON, SHAW, 1973; LAINSON, SHAW, 1979; LAINSON et al., 1981a; LAINSON et al. 1981b; BRANDÃO-FILHO et al., 1994; VASCONCELLOS et al., 1994; BRANDÃO-FILHO et al., 2003).

Infecções por *Leishmania* foram descritas em várias espécies de animais silvestres, sinantrópicos e domésticos (canídeos, felídeos e equídeos) (GRIMALDI, TESH, 1993). Nas primeiras décadas do século passado procurou-se descobrir em todo o mundo reservatórios primários de protozoários do gênero *Leishmania*, considerando o reservatório como um sistema ecológico no qual o agente infeccioso persiste indefinidamente (ASHFORD, 1996; ASHFORD, 2000).

No estado do Paraná, a ocorrência de infecção de cães, equinos e animais silvestres

por *Leishmania* sp. está intimamente relacionada ao surgimento de casos em humanos (SILVEIRA et al. 1996; LONARDONI et al. 2006; VOLTARELLI et al. 2009; MEMBRIVE et al. 2012).

As alterações do meio ambiente decorrentes das ações antrópicas para a exploração de recursos naturais e agricultura modificaram a epidemiologia da LT viabilizando o surgimento de novas áreas endêmicas (LAINSON, SHAW, 1998).

A diversidade de parasitos e a negligência em relação as leishmanioses pelos serviços de saúde pública mostram a importância de estudos eco-epidemiológicos, envolvendo hospedeiros, reservatórios e flebotomíneos, vetores de *Leishmania* spp.

INTERAÇÃO DE PARASITOS, VETORES E RESERVATÓRIOS EM ÁREAS ANTRÓPICAS

No estado do Paraná o ciclo de *Leishmania* em focos naturais tem relação íntima com áreas florestais naturais próximas às áreas de produção agropecuária tradicional. As áreas com maior intensidade de casos localizam- se sobretudo nas bacias dos rios Ivaí e Pirapó, onde há florestas residuais nativas (LIMA et al. 2002; MONTEIRO et al. 2008; MONTEIRO et al. 2009) (Figura 2). Na zona urbana, a doença ocorre em áreas com fragmentos preservados de cobertura florestal, a exemplo dos municípios de Maringá (TEODORO et al. 1998; LIMA et al. 2002) e Cianorte (LIMA et al. 2002). Apesar da substituição da vegetação original de florestas pelas culturas de café, soja, milho, algodão, cana-de-açúcar e por pastagens, a persistência da LT no Paraná é fruto de adaptações bem sucedidas de vetores e reservatórios de parasitos em áreas antrópicas (LIMA et al. 2002).

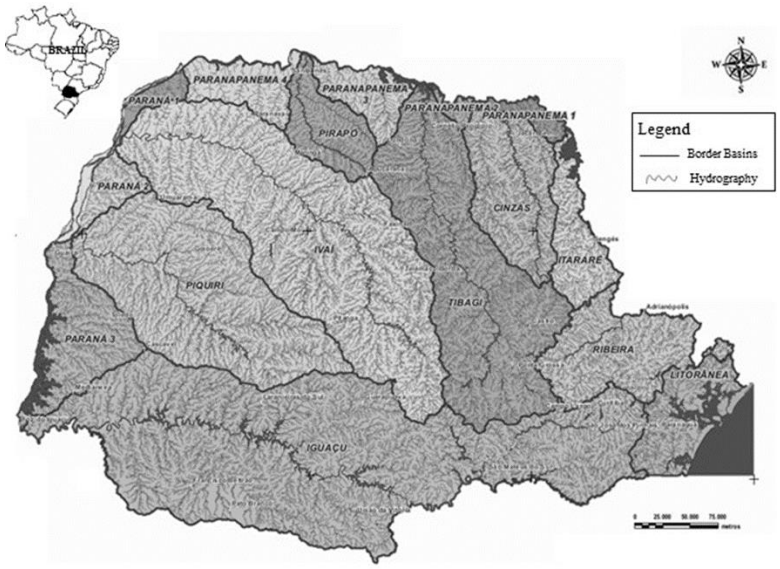

Figura 2. Bacias Hidrográficas do Paraná, Brasil. Fonte: SEMA (2007)

EPIDEMIOLOGIA

LT ocorre na América, na bacia do mediterrâneo, no Oriente Médio e na Ásia Central (OMS, 2017). Afeganistão, Brasil e Irã são os países de maior ocorrência, com mais de dois terços dos casos (OMS, 2017). Em países como Argélia, Colômbia e Síria, estima-se que há de 700 mil a 1,3 milhões de novos casos anuais (Figura 3) (OMS, 2017). Na América Latina o número de casos de LT se mantém elevado, especialmente no Brasil, onde foram registrados 587.962 casos, no período de 1990 a 2011 (BRASIL, 2016).

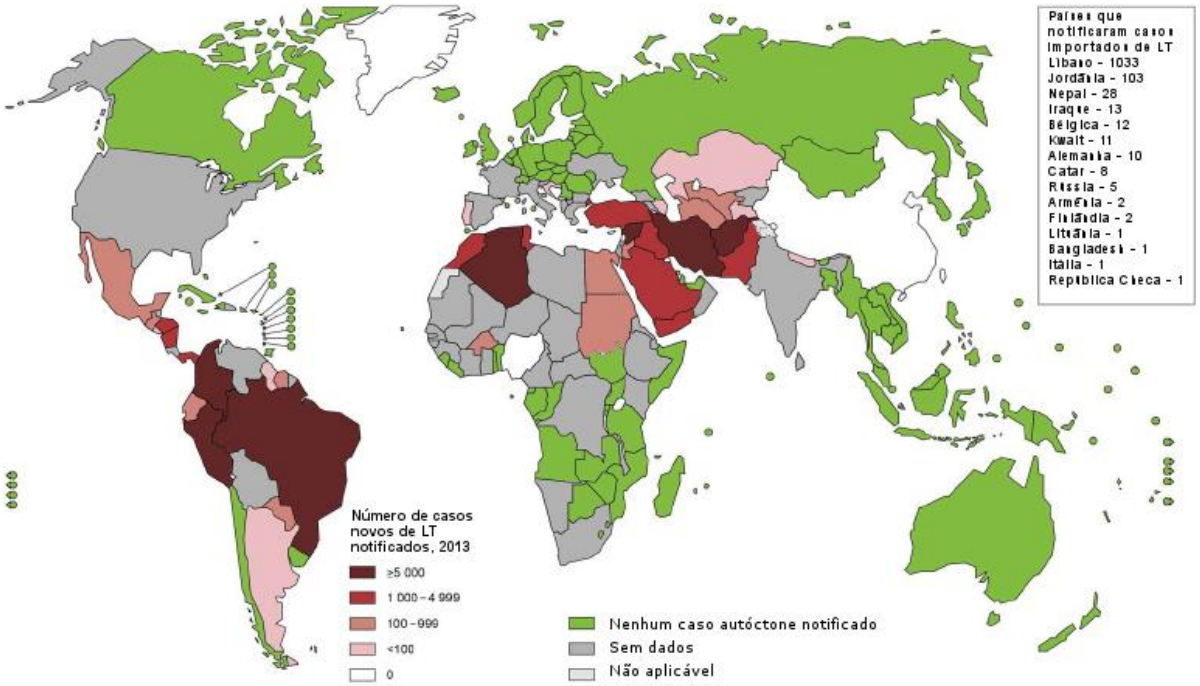

Figura 3. Situação da endemia de leishmaniose tegumentar mundial, 2013. Fonte: OMS (2017)

No Brasil, a partir da década de 80, houve crescimento extraordinário do número de casos com maior número de notificações em 1994 e 1995 (BRASIL, 2007) (Figura 4A). No período de 1985 a 2016, a média anual de casos registrados foi de 19.521, observando-se a estabilidade da LT como endemia até 2013, com queda brusca de casos a partir deste ano (Figura 4B) (BRASIL, 2016).

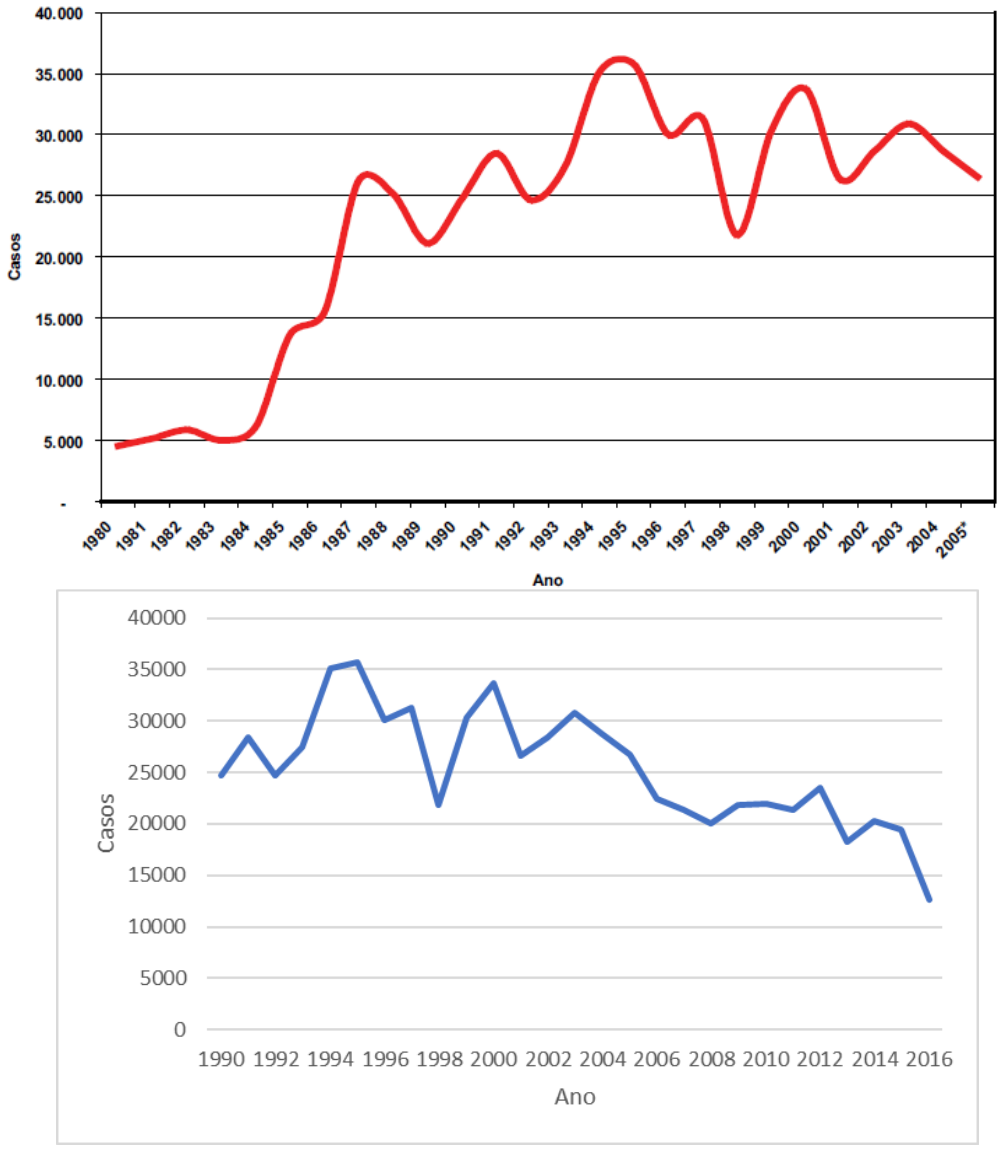

Figura 4. Casos notificados de leishmaniose tegumentar americana, Brasil – 1980 a 2016. Fonte: BRASIL (2017)

Na região sul do Brasil, de 1990 a 2011, ocorreram 13.161 casos de LT, dos quais 94,9% no estado do Paraná (BRASIL, 2016). A incidência de casos de LT no Paraná foi relatada no início do século XX no norte, oeste e sudoeste deste estado (LUZ et al., 2000). Períodos epidêmicos da doença ocorrem entre 1930 e 1950, durante o processo de colonização do interior do estado em decorrência da introdução da monocultura de café (LUZ et al., 2000). As campanhas de controle da malaria com o uso de inseticidas clorados contribuiram para a diminuição de casos de LT no estado na década de 1950. Contudo, a doença retornou na década de 1980 e persiste de forma endêmica em áreas na zona rural e urbana (SILVEIRA et al., 1996; SILVEIRA et al., 1999; LIMA et al., 2002).

ANÁLISES ESPACIAIS

As análises espaciais utilizadas na Saúde Pública auxiliam na compreensão dos padrões espaciais de risco humano para a exposição a vetores e seus patógenos associados (MAGALHÃES, 2012). Estas análises pressupõem que os dados mais próximos e espacialmente correlacionados tenham um comportamento semelhante entre si (CÂMARA et al., 2002). A análise geoestatística permite a confecção de mapas de risco de ocorrência de doenças (MAGALHÃES, 2012).

Um dos principais testes para detectar a autocorrelação espacial é o Índice de Moran, definido como (ANSELIN, 2013):

$$I_i = \frac{(y_i - \bar{y}) \sum_{j=1}^{n} w_{ij} (y_j - \bar{y})}{\frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n}}$$

em que:

y_i, y_j são as observações coletadas nos pontos i e j respectivamente;

$\bar{y}$ é o valor médio;

w_{ij} é o componente de vizinhança espacial;

n é o tamanho da amostra.

Este teste permite verificar se há interferência do fator espacial em nível geral, mas não determina os fatores que influem na ocorrência de agravos em saúde. Após verificar a existência de autocorrelação espacial, determina-se com a modelagem do fenômeno em estudo com base no seu objetivo.

INDICADORES LOCAIS DE ASSOCIAÇÃO ESPACIAL

Mesmo que o Indice Global de Moran indique devidamente o comportamento espacial, ele gera apenas um valor de associação para toda a área estudada (ANSELIN, 2013).

Quando há um número elevado de áreas, pode ser que haja a necessidade de examinar com mais detalhes a diferença entre elas, identicando se há locais com dependência espacial mais acentuada (ANSELIN, 2013).

Os testes locais avaliam a covariância entre um determinado polígono e uma certa vizinhança definida em função de uma distância *d* (ANSELIN, 2013).

Dado um modelo de vizinhança pré-estabelecido, a estatístistica espacial local quantifica os graus de associação espacial que cada localização do conjunto amostral está submetida. Tal resultado é possível a partir de indicadores locais de associação espacial (ANSELIN, 2013).

Segundo Anselin (2013), a soma de todos os LISAs (valores de autocorrelação local) é diretamente proporcional aos valores de autocorrelação global, apontando que o indicador local seja uma decomposição do global.

Os LISAs permitem que os indicadores globais se decomponham em contribuições individuais que indicam porções territoriais de não estacionariedade e identificação de *clusters*, pois geram um índice para cada área, evidenciando as que possuem maiores semelhanças (ANSELIN, 2013).

Um dos LISAs mais utilizados é o Indice Local de Moran (Ii):

$$I_i\,(d) = \frac{(x_i - \overline{x})}{s^2} \sum w_{ij}(d)\,(x_i - \overline{x})$$

x_i são as amostras coletadas nos pontos i;
$\bar{x}$ é o valor médio;
w_{ij} é o componente de vizinhança espacial;
s^2 é a variância

O índice local de Moran determina a autocorrelação espacial a partir do produto dos desvios em relação à média como uma medida de covariância. Quando o cálculo resulta em um valor alto, interpretamos que existe alta probabilidade de existirem locais de associação espacial em regiões com altos ou baixos valores associados. Com essa análise podemos determinar áreas dentro de uma determinada região de estudo, seja município ou estado, com maiores ou menores risco de ocorrência de determinada doença ou agravo (ANSELIN, 2013).

MODELO SIMULTÂNEO AUTOREGRESSIVO

O modelo SAR (*simultaneous autoregressive model*) utiliza uma regressão de valores de outras áreas para modelar a dependência espacial (BIVAND et al., 2008). Isto significa que o erro ℓ é modelado considerando sua dependência com os outros erros, isto é,

$$e_i = \sum_{i=1}^{m} b_{ij} e_i + \varepsilon_i$$

em que
b_{ij} são valores que representam a dependência espacial entre áreas;

ε_i representa o erro residual que é considerado independente e identicamente distribuído segundo uma normal com média zero e variância constante.

Este modelo é conhecido como modelo misto de regressão espacial, o que significa a possibilidade de análise de uma variável independente em relação a outras variáveis dependentes (ex: idade dos pacientes, condições socioeconômicas, vegetação etc.) na autocorrelação espacial de determinado agravo.

JUSTIFICATIVA

A dimensão da distribuição geográfica e a densidade de casos da LT nos circuitos Paraná-Paranapanema e Ribeira de produção da doença, o modelo de colonização e ocupação do espaço rural, são fortes argumentos para a análise ininterrupta da situação deste agravo no estado do Paraná. O que pode ser realizado com a incorporação de técnicas de geoprocessamento e análise espacial nos estudos de epidemiologia de agravos à saúde (MAGALHÃES, 2012). O geoprocessamento é um conjunto de técnicas de coleta, tratamento e manipulação de dados geográficos que exibem informações geograficamente referenciadas, tornando-se uma ferramenta valorosa na análise da dinâmica de agravos em saúde pública e suas relações com o meio ambiente (MEDRONHO et al., 2003; MAGALHÃES, 2012).

A análise de dados de geoprocessamento de áreas de ocorrência de LT com diferentes graus de antropia ambiental no estado do Paraná, pode proporcionar a produção de mapas de risco para auxiliar de forma mais eficaz o conhecimento e o controle desta zoonose no estado.

OBJETIVOS

GERAL

Estabelecer as áreas de risco da LT associadas aos diferentes graus de antropia ambiental no estado do Paraná

ESPECÍFICOS

Avaliar as áreas de risco da LT no período de 2001-2015, utilizando métodos de análise estatística do geoprocessamento de dados;

Analisar o risco da ocorrência de LT em áreas de vegetação remanescente, nos anos de 2012-2013, utilizando métodos de análise estatística do geoprocessamento de dados;

Identificar as áreas com maior risco de ocorrência de LT no estado do Paraná.

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CAPITULO II

Artigo 1:
“SPATIAL DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN THE STATE OF PARANÁ, BRAZIL”

Spatial distribution of cutaneous leishmaniasis in the state of Paraná, Brazil

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Abstract

The geographic distribution of cutaneous leishmaniasis (CL) makes it a disease of major clinical importance in Brazil, where it is endemic in the state of Paraná. The objective of this study was to analyze the spatial distribution of CL in Paraná between 2001 and 2015, based on data from the Sistema de Informação de Agravos de Notificação (Information System for Notifiable Diseases) regarding autochthonous CL cases. Spatial autocorrelation was performed using Moran's Global Index and the Local Indicator of Spatial Association (LISA). The construction of maps was based on categories of association (high-high, low-low, high-low, and low-high). A total of 4,557 autochthonous cases of CL were registered in the state of Paraná, with an annual average of 303.8 (± 135.2) and a detection coefficient of 2.91. No correlation was found between global indices and their respective significance in 2001 (I = -0.456, p = 0.676), but evidence of spatial autocorrelation was found in other years (p< 0.05). In the construction and analysis of the cluster maps, areas with a high-high positive association were found in the Ivaí-Pirapó, Tibagi, Cinzas-Laranjinha, and Ribeira areas. The state of Paraná should keep a constant surveillance over CL due to the prominent presence of socioeconomic and environmental factors such as the favorable circumstances for the vectors present in peri-urban and agriculture áreas.

Keywords: Leishmaniasis; Epidemiology; Zoonoses; Spatial Analysis

Introduction

Leishmaniasis is a globally distributed disease. Approximately 350 million people are currently at risk of contracting at least one of its variants [1]. Brazil had an annual average of 26,965 registered cases of cutaneous leishmaniasis (CL) from 1993 to 2012, with an average detection coefficient of 15.7 cases for every 100,000 inhabitants [2]. Throughout this period, an increasing trend was observed, with higher coefficients in 1994 and 1995, 22.83 and 22.94 cases for every 100,000 inhabitants, respectively [2]. When analyzing the evolution of CL in Brazil, one noticeable factor is its geographical expansion. At the beginning of the 1980s, autochthonous cases were registered in 19 states. By 2003, every state in the country had registered cases of CL [2]. Since the early 1900s, human cases of CL have been registered in northern, western, and southeastern regions of the state of Paraná. In the northern region of Paraná, the disease reached epidemic proportions between the 1930s and 1950s when the area was experiencing significant immigration [3]. The incidence dropped drastically during the 1950s as a direct result of public campaigns for the eradication of malaria and the use of insecticides [3]. However, since the 1980s, the incidence of CL has returned to endemic proportions in the state of Paraná [2,4].

In Brazil, *Leishmania (Viannia) braziliensis, L. (Leishmania) amazonensis*, and *L. (V.)guyanensis* have been the most frequent causes of CL in humans [2]. In Paraná, CL is directly linked to wild transmission cycles of the parasite in natural foci that persist in forest preserve areas and traditional agricultural production zones [5-7]. Cutaneous leishmaniasis persists in the state despite the replacement of natural vegetation with corn, cotton, and pasture plantings, affecting individuals of all age groups and both genders [5-7]. Anthropogenic actions that affected the environment and increased urbanization and socioeconomic pressure may have contributed to an increase in endemic areas and outbreaks in urban areas [5]. In areas that have been modified by human activity, CL has been found in environmental preservation areas

with small patches of forest, such as the cities of Maringá [5,8] and Cianorte [5].

Understanding spatial patterns with the use of human risk geoprocessing techniques is important for the proper guidance of prevention, surveillance, and control measures [9-11], based on the assumption that spatially related data samples within close proximity to each other possess similar behavior. The use of geoprocessing techniques and statistical spatial analysis enables the creation of maps that detail the risk of occurrence of CL. Based on these analyses, associations between cases of CL and different degrees of anthropogenic activity can be determined in areas where there is notification of the disease, with the goal of identifying possible patterns between such areas [10]. The aim of the present study was to use statistical spatial analysis in the state of Paraná to evaluate the dynamics of CL occurrence from 2001 to 2015 in an attempt to support planning control measures that can effectively mitigate the impact of the disease on the population.

Materials and methods

Study area

The state of Paraná is in southern Brazil (22º30'58" and 28º43'00" S; 48º05'37" and 54º37'08" W). It has an area of 199,307.945 km^2 and an estimated population of 11,163,018, with a demographic density of 52.40 inhabitants per square kilometer in 2015 [12-13]. Paraná has 399 municipalities that are distributed into 10 macro regions (i.e., geopolitical subdivisions that encompass several municipalities with economic and social similarities) and 39 micro regions (i.e., a group of neighboring municipalities) [12-13].

Paraná has three distinct climatic groups, according to the Köppen climate classification system: (1) Humid Subtropical Climate – Mesothermal (Cfa), with an average high temperature of 22ºC that can reach 40ºC in the north, west, and Ribeira river valley and an average low

temperature of 18ºC (this is the most widespread type of climate in the state, (2) Temperature Oceanic Climate – Mesothermal (Cfb), with an average temperature of 18-22ºC, and (3) Tropical Rainforest Climate – Megathermal (Af), which is restricted to the coastal strip and has an average temperature above 18ºC [14].

Data collection

To analyze the spatial distribution of autochthonous cases of CL in the state of Paraná, we used data from the Sistema de Informação de Agravos de Notificação (SINAN; Information System for Notifiable Diseases) from January 2001 to December 2015. To calculate the detection coefficient (autochthonous cases per 100,000 inhabitants), we used the estimated annual population and the territorial area of each municipality, based on the Instituto Brasileiro de Geografia e Estatística (Brazilian Institute for Geography and Statistics) [12-13]. We gathered information on gender, age, clinical form of CL, and proportion of CL patients that achieved clinical cure. In the present study, we focused on municipalities with detection coefficients >10.0 because the highest risk for CL transmission in these areas.

Statistical analysis

The spatial analysis was conducted in three stages. In the first stage, a test was performed to detect spatial autocorrelation and verify global spatial dependency against the incidence of autochthonous cases of CL [15]. In the second stage, the Local Indicator of Spatial Association (LISA) was employed to analyze local spatial association, which produces a specific value for each municipality and allows the identification of clusters of municipalities with local similarities in terms of the incidence of CL [15]. In the third stage, maps were constructed by category, with two possible classes of direct association (high-high and low-low) and two possible classes of negative association (high-low and low-high) [15].

To detect spatial autocorrelation, Moran's Global Index (Moran's I) was used, defined as:

$$I_i = \frac{(y_i - \bar{y})\sum_{j=1}^{n} w_{ij}(y_j - \bar{y})}{\frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n}}$$

where y_i, y_j are the samples collected at points i and j, respectively, $\bar{y}$ is the mean value, w_{ij} is the spatial neighboring component, and n is the size of the sample.

For LISA, Moran's Local Index was used, defined as:

$$I_i(d) = \frac{(x_i - \overline{x})}{s^2}\sum w_{ij}(d)\,(x_i - \overline{x})$$

where x_i is the sample collected at point i, $\bar{x}$ is the mean value, w_{ij} is the spatial neighboring component, and s^2 is the variance.

All of the statistical analyses were performed with software R environment confidence interval of 95% [16] and with package "spdep" [17-18]. This package provides a collection of functions to create spatial weights matrix objects from polygon contiguities, from point patterns by distance and tessellations, for summarizing these objects, and for permitting their use in spatial data analysis, including regional aggregation by minimum spanning tree.

Results

From 2001 to 2015, 4,557 cases of CL were diagnosed in the state of Paraná, with an average annual case rate of 303.8 (±135.2), a detection coefficient of 2.91, and a density of

0.023 cases per km^2. The year with the highest number of cases was 2003 (629 cases) with a detection coefficient of 6.35 (Fig 1). Males between 20 and 59 years of age were most affected, with a predominance of the cutaneous clinical form and evolution of the majority of the treated cases to clinical cure (77.77%) (Table 1).

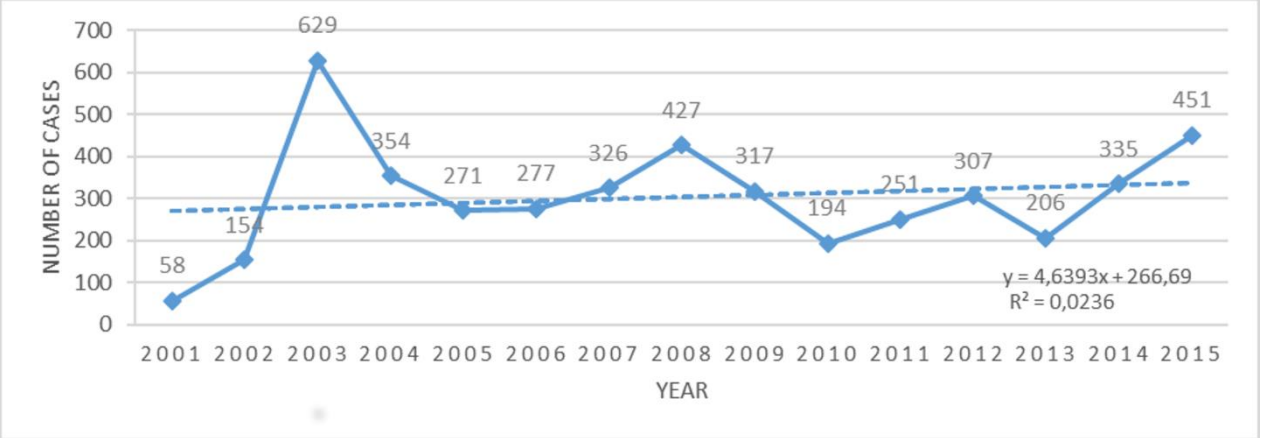

Fig 1. Distribution of cutaneous leishmaniasis cases in the state of Paraná, Brazil, from 2001 to 2015.

TABLE 1 Demographic and clinical characteristics of cutaneous leishmaniasis cases in the state of Paraná, Brazil, from 2001 to 2015 by the chi-squared test.

Characteristic	n	%	*p*-value
Gender			<0.001
Male	3,137	68.84	
Female	1,420	31.16	
Age Group (years)			<0.001
>1	33	0.72	
1-4	76	1.67	
5-9	184	4.04	
10-14	228	5.00	
15-19	281	6.17	
20-39	1,490	32.70	
40-59	1,435	31.49	
60-64	278	6.10	
65-69	228	5.00	
70-79	246	5.40	
≥80	78	1.71	
Clinical form			<0.001
Cutaneous	4,145	90.96	
Mucocutaneous	410	9.00	
Not informed	2	0.04	
Case outcome			<0.001
Clinical cure	3,544	77.77	
Abandonment of treatment	107	2.35	
Death related to CL	7	0.15	
Death of other cause	62	1.36	
Transfer	44	0.97	
Change diagnosis	44	0.97	
Not informed	749	16.44	

The occurrence of CL cases was verified in 268 municipalities (61.17%); of these, eight municipalities (2.99%) had a detection coefficient ≥ 71.0, 35 municipalities (13.06%) had a detection coefficient between 10.0 and 71.0, 95 municipalities (35.45%) had a detection coefficient between 2.5 and 10.0, and 130 municipalities (48.51%) had a detection coefficient <2.5.

Among the 268 municipalities in the state with registered cases of CL, the following were especially notable: 341 in Londrina (7.48%), 331 in Cianorte (7.26%), 279 in Cerro Azul (6.12%), 232 in Jussara (5.09%), 184 in Terra Boa (4.04%), 177 in Bandeirantes (3.88%), 158 in Adrianópolis (3.47%), 134 in Umuarama (2.84%), 111 in Japurá (2.44%), and 86 in Maringá

(1.89%). Altogether, these municipalities comprised 44.61% of all cases of CL in the study period. The municipalities with the highest detection coefficients were Jussara (237.47), Adrianópolis (165.03), Cerro Azul (108.29), Ivatuba (108.11), and Japurá (89.35) (Table 2).

TABLE 2 Number and detection coefficient of cases of cutaneous leishmaniasis in municipalities with a detection coefficient >10.0 in the state of Paraná, Brazil, from 2001 to 2015.

Municipality	No. of cases	Detection coefficient*	Municipality	No. of cases	Detection coefficient*
Abatiá	22	19.13	Japurá	111	89.35
Adrianópolis	158	165.03	Jussara	232	237.47
Araruna	23	11.44	Lobato	10	15.29
Ariranha do Ivaí	9	23.58	Munhoz de Melo	6	11.21
Bandeirantes	177	35.85	Nova Tebas	11	10.58
Cambira	13	12.17	Pinhalão	25	26.49
Cândido de Abreu	54	20.88	Porto Rico	5	13.68
Carlópolis	82	39.69	Prudentópolis	81	11.21
Cerro Azul	279	108.29	Rio Bom	13	26.22
Cianorte	331	32.89	Rio Bonito do Iguaçu	75	32.67
Colorado	50	15.00	Sabaúdia	11	12.57
Conselheiro Mairinck	9	16.66	Santa Amélia	9	14.84
Corumbataí do Sul	12	19.53	São Carlos do Ivaí	12	12.65
Cruzeiro do Sul	8	11.56	São Jerônimo da Serra	67	39.26
Doutor Camargo	74	85.12	São Jorge do Ivaí	72	80.54
Doutor Ulysses	37	40.48	São Jorge do Patrocínio	39	45.26
Enéas Marques	12	13.24	São Tomé	49	61.74
Engenheiro Beltrão	71	33.81	Terra Boa	184	79.61
Grande Rios	14	13.01	Tomazina	47	35.25
Icaraíma	38	28.01	Tuneiras do Oeste	66	52.08
Itambaracá	32	32.68	Uniflor	7	19.47
Ivatuba	48	108.11	**Total**	2,685	38.69

*Cases per 100,000 inhabitants.

The results of the global indices and their respective significance revealed no correlation in 2001 (I = -0.456, $p = 0.676$). In subsequent years, statistical evidence of a spatial correlation (Table 3) allowed the construction of LISA cluster maps (Fig 2). Fig 2 shows a high-high cluster area in municipalities in the Lower Ivaí Basin in each year from 2002 to 2015. High-high clusters were also observed in other basins, such as those of the Pirapó, Tibagi, and Ivaí Rivers. These were smaller in 2010 and 2014 and not evident in 2002. In the Ribeira River basin, a high-high association was found during the study years, except in 2002, 2005, 2010,

2011, 2012, and 2014. In 2010, Curitiba was a high-high cluster area, with a direct association for the occurrence of CL. In 2003, the highest number of municipalities with a direct association was found in the Ivaí River Basin and Pirapó River Basin. In the municipalities of the Lower Iguaçu River Basin, a positive association was found only in 2005 (Fig 2).

TABLE 3 Detection coefficient, Global Moran Index, and respective significance of cutaneous leishmaniasis in the state of Paraná, Brazil, between 2001 and 2015.

Year	Detection coefficient	Global Moran Index	*p*
2001	0.60	-0.456	0.676
2002	1.57	3.028	<0.01
2003	6.35	7.344	<0.01
2004	3.49	9.276	<0.01
2005	2.64	2.689	<0.01
2006	2.67	4.856	<0.01
2007	3.17	11.120	<0.01
2008	4.03	11.145	<0.01
2009	2.97	11.084	<0.01
2010	1.86	9.227	<0.01
2011	2.39	8.305	<0.01
2012	2.90	9.272	<0.01
2013	1.87	6.473	<0.01
2014	3.02	11.946	<0.01
2015	4.04	8.376	<0.01

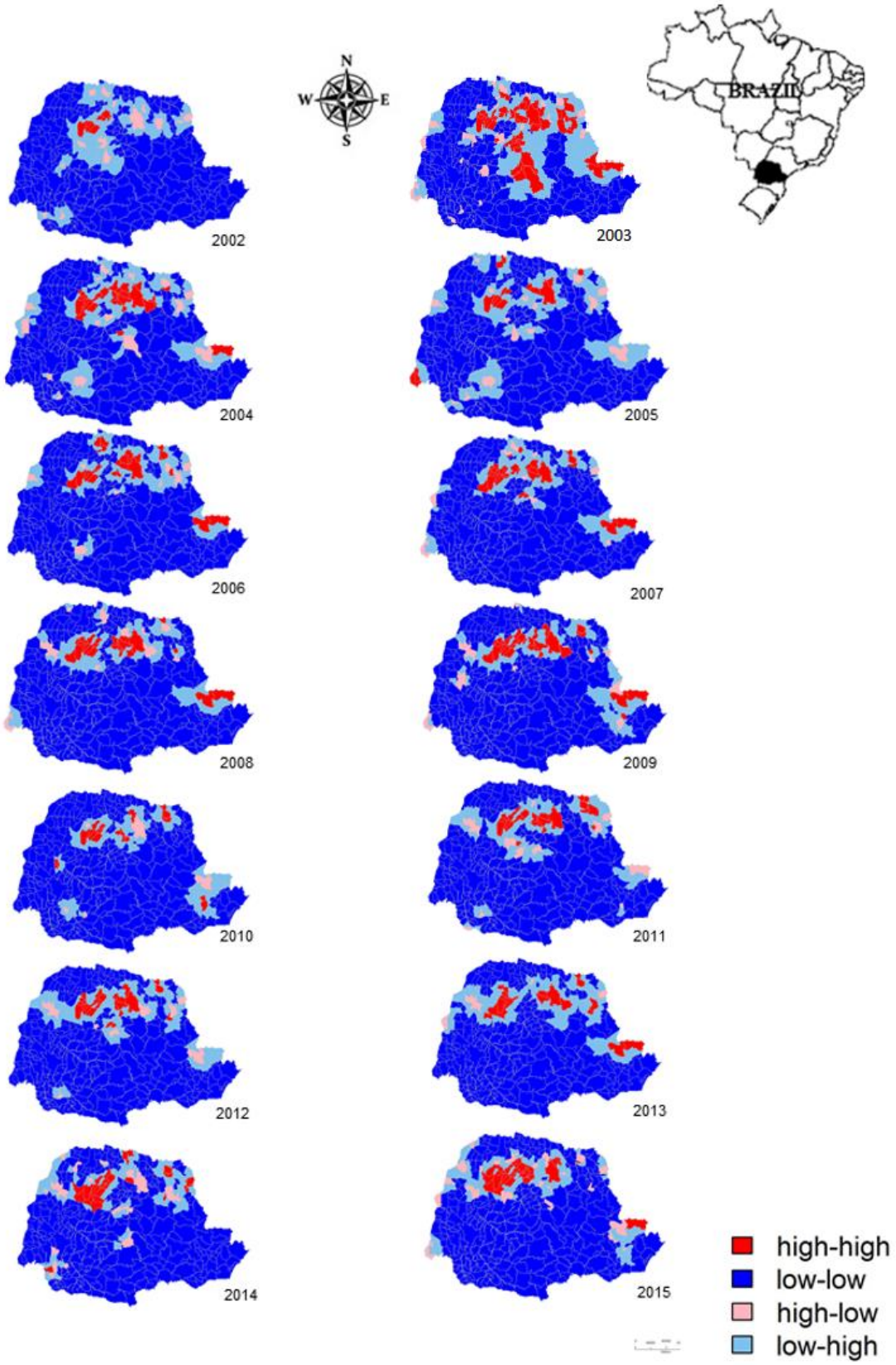

Fig 2. LISA cluster maps detailing the incidence of autochthonous cases of cutaneous leishmaniasis from 2002 to 2015. Darker areas indicate direct spatial autocorrelation. Lighter areas indicate negative autocorrelation.

Discussion

The spatial distribution of CL in all Brazilian states shows the importance of this disease in the country [2]. The spatial distribution of CL demonstrates the significance of this disease throughout Brazil, with an increase in the number of cases in the 1980s and 1990s [2]. In Paraná, the disease has been registered in areas of ancient colonization, contrary to the expectation that the increase in human activities in the environment would result in the elimination of natural foci of CL [2].

The majority of infected individuals in Paraná during the study period were male and located in municipalities where the main economic activity is agriculture [6,7]. The most affected age group was 20-59 years, probably related to the agriculture work or recreational activities like fishing near riparian forests of rivers and streams where the enzootic cycle of *Leishmania* remains [5]. This was also observed in the municipality of Teodoro Sampaio in the state of São Paulo, Brazil [19], and in the country of Iran [20], which have features that are distinctive from Paraná, signifying that men may engage in behaviors that can lead to a higher risk of CL. Previous studies reported that the proportion of infected individuals is similar between agricultural and domestic workers [6,7]. The majority of urban residents acquired CL in Paraná during the study period in the rural area suggesting that pendulum migration is an important risk factor for CL in mesoregions north central, western center and northwest. In the state of Paraná [6]. The considerable number of women and children with CL that have been identified in studies in Paraná, including the present study, corroborate this assessment [6,7]. The number of female cases (31.15%), although less than males, is notable. Such cases appear to be more related to activities that are connected with agricultural work and the construction of residences and domestic animal shelters that are in close proximity to modified native forest where the environment is fresher and more pleasant [7].

Although cases of CL were registered in 268 municipalities, the municipalities with the

highest detection coefficients were concentrated in the Ivaí-Pirapó CL hub, which is part of the Paraná-Paranapanema CL production circuit [7]. Examples of this are Jussara and Cianorte, which have large areas of moderately or highly altered residual forest and also secondary forests [5-7]. In the Alto Ribeira hub within the Ribeira circuit [7], the municipalities of Adrianópolis and Cerro Azul had elevated CL detection coefficients.

Londrina is part of the Ivaí-Pirapó hub. Although the municipality of Londrina had a detection coefficient <10 because of its larger population, it was responsible for the highest number of cases of CL between 2001 and 2015. A cluster of municipalities with high detection coefficients was identified in the Cinzas-Laranjinha area (Parana-Paranapanema circuit), one example of which is the municipality of Bandeirantes. Cases of CL were also registered in the municipalities of Cândido de Abreu and Prudentópolis in the Tibagi area (Paraná-Paranapanema circuit). A notable occurrence of cases was observed in the municipalities of the Lower Iguaçu area (Paraná-Paranapanema circuit), such as Rio Bonito do Iguaçu and Enéas Marques. Areas of high anthropogenic impact that is related to agriculture, especially corn, soybeans, sugar cane, and pasture, focus cases of CL in Paraná state [5].

The LISA map analysis revealed that only the municipalities of Jussara and Cianorte in the Ivaí-Pirapó area maintained a high-high association from 2002 to 2015. These municipalities play a major role in the production of CL in this area [7]. Previous studies have investigated *Leishmania* infection in dogs and wild animals in these areas [21-25]. The high number of phlebotomine sandflies in areas of altered native forest in the Ivaí-Pirapó area [3,26-28] may partially explain the persistence of the high-high cluster throughout the duration of the study period. Interestingly, the sandfly species *Nyssomyia neivai* (Pinto), *Nyssomyia whitmani* (Antunes & Coutinho), and *Migonemyia migonei* (França) were identified as *Leishmania* vectors [3,29,30], which are widely distributed in the state of Paraná [31]. In 2003, the high-high association covered all CL production areas in Paraná, with the exception of the Lower

Iguaçu area.

The spatial analysis of the SINAN data from 2001 to 2015 allowed visualization of the local and global distribution, cluster formation, and spatial instability and identification of outliers of CL throughout the state of Paraná [32]. Beyond the use of georeferencing instruments, it is important to further investigate the migration of human populations and local conditions that influence the risk for CL in the referenced areas [33].

The public health surveillance should take into account the differences between the transmission patterns of each locality and the identified high-risk cluster in developing actions to mitigate the properties of the zoonosis in the state of Paraná. Moreover, the disease affects specific areas, such as Alto Ribeira, Ivaí-Pirapó, Tibagi, and Cinzas-Laranjinha. This suggests that health authorities need to provide information and develop campaigns regarding the importance of early diagnosis and treatment of CL, with the goal of reducing the emergence of new cases and preventing mucocutaneous cases of the disease. Moreover, the control of sandflies is essential to block the spread of the disease.

Conclusion

The state of Paraná should keep a constant surveillance over cutaneous leishmaniasis due to the prominent presence of socioeconomic and environmental factors such as the favorable circumstances for the vectors present in peri-urban and agriculture areas.

Ivaí-Pirapó, Tibagi, Cinzas-Laranjinha, and Ribeira areas form the four-major hot spot CL areas in the state of Paraná.

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Artigo 2:

"INFLUENCE OF NATIVE VEGETATION ON THE SPATIAL DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN PARANÁ, BRAZIL"

INFLUENCE OF NATIVE VEGETATION ON THE SPATIAL DISTRIBUTION OF CUTANEOUS LEISHMANIASIS IN PARANÁ, BRAZIL

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ABSTRACT

Background: Cutaneous leishmaniasis (CL) is endemic in the Paraná state of Brazil. Anthropogenic activities, space element and the remaining forests influence the health-disease process.

Objective: To analyze the influence of the remaining native vegetation on the occurrence of CL cases in the state of Paraná, using georeferencing.

Methods: To achieve the objectives, we used global testing for spatial autocorrelation with simultaneous autoregressive model (SAR). The regression was based on the CL coefficient (cases/100,000 inhabitants), as a function of the natural vegetation cover (km^2), percentage of natural vegetation cover, altitude, total number of cases and spatial density per km^2, with the location data of the Paraná state municipalities and the detection coefficient (cases/100,000 inhabitants) of autochthonous cases of CL obtained from the Sistema de Informação de Agravos de Notificação (SINAN; Information System for

Notifiable Diseases) for the years 2012 and 2013. We obtained data on the remaining forests from the Fundação SOS Mata Atlântica (SOS Mata Atlântica Foundation) and Instituto Nacionais de Pesquisas Espaciais (INPE; National Institute of Space Research). Findings: Spatial regression of the coefficient of detection revealed statistical significance only for spatial density (Z = 22,1359, p <0,05), while the results for the other variables were not significant.

Main conclusions: concentration of LT cases occurs at the production poles of this disease, where there is also a concentration of native vegetation, represented mainly by riparian forests

Keywords: Cutaneous Leishmaniasis; Zoonoses; Environmental Health; Spatial Analysis; Spatial Regression

INTRODUCTION

Cutaneous leishmaniasis (CL) occurs in 97 countries of America, Europe, Africa and Asia, with an annual registry of 0.7 to 1.3 million new cases (WHO 2017). This disease is one of the six most significant infectious diseases in the world, due to its high detection coefficient and ability to cause deformations in patients (Brazil 2016a). In Brazil, from 1993 to 2016, the annual average number of CL cases was 22,140, with a detection coefficient of 9.8 cases/100,000 inhabitants (Brazil, 2016a, Brazil 2017a). CL is endemic in Brazil. In the early 1980s, autochthons cases were registered in 19 states, while in 2003, there are registered cases in every state of the country (Brazil 2016a).

According to Luz et al. (2000), between 1930 and 1950, CL reached epidemic proportions to the north of Paraná during the colonization period, due to the replacement of native vegetation with coffee plantations. In the 1950s, the incidence of this zoonosis reduced sharply, due to the indirect effect of the malaria control campaigns with

chlorinated insecticides (Luz et al. 2000). However, since 1980, CL has been endemic in Paraná (Silveira et al. 1996, Silveira et al. 1999, Lima et al. 2002). From 1980 to 2016, 16,464 cases were reported in the northern, western and southwestern parts of the state (Brazil 2017a, Brazil 2017b).

In Paraná state, the natural *Leishmania* cycle has an intimate relationship with the natural forest areas close to the traditional agricultural production areas. Areas with the highest intensity of cases are located in the basins of the Ivaí and Pirapó rivers, where there are native residual forests (Lima et al. 2002, Monteiro et al. 2008, Monteiro et al. 2009), as well as in the urban areas of Maringá (Teodoro et al. 1998, Lima et al. 2002) and Cianorte (Lima et al. 2002). Despite the substitution of native forest vegetation for coffee, soybean, corn, cotton and pasture crops, the persistence of CL in Paraná is the result of the successful adaptation of vectors and reservoirs of parasites in areas to the effects of human activity (Lima et al. 2002).

Data on the occupation of agrarian and urban spaces, vegetation cover and the geographical distribution of CL, allows the formulation of hypotheses and the planning of actions to be performed by the public health services towards the control of CL, especially its vector (Monteiro et al. 2008, King et al. 2004). Therefore, the factors that influence the geographical distribution of CL were submitted for statistical analysis using georeferencing, which will be very valuable in the planning of health initiatives (Medronho et al. 2003, Eisen and Eisen 2008, Magalhães 2012). The objective of this work was to use statistical analysis through georeferencing to evaluate the relevance of the association between residual native vegetation data and CL cases, as a risk factor for the occurrence of this disease in the state of Paraná.

METHODOLOGY

Description of Paraná state

The state of Paraná is in southern Brazil (22º30'58" and 28º43'00" S; 48º05'37" and 54º37'08" W). It covers an area of 199,307.945 km^2, with 399 municipalities that are distributed across 10 macro-regions and 39 micro-regions. During the study period, the estimated population was 10,997,465, with a demographic density of 55.18 inhabitants/km and a human development index (HDI) of 0.749 (Brazil 2010, Brazil 2016b).

Paraná has three distinct climatic groups, according to the Köppen climate classification system: Tropical Rainforest Climate—Megathermal (Af), which is restricted to the coastal strip, has an average temperature higher than 18 ºC; Humid Subtropical Climate—Mesothermal (Cfa) has an average high temperature of 22 ºC that can reach up to 40 ºC in the north, west, and the Ribeira river valley, while the average low temperature is 18 ºC (this is the most widespread type of climate in the state); and Temperature Oceanic Climate—Mesothermal (Cfb), which has an average temperature of 18–22 ºC (Paraná, 2002).

Data collection

For analyzing the influence of native vegetation on autochthonous cases of CL in the state of Paraná, data was obtained from Sistema de Informação de Agravos de Notificação (SINAN; Information System for Notifiable Diseases) for the period from January 2012 to December 2013 (Brazil, 2015). For the coefficient of detection calculation (autochthonous cases per 100,000 inhabitants), spatial density (cases per km^2), altitude, and territorial area, we used population and territorial data of each municipality, based on the Instituto Brasileiro de Geografia e Estatística (Brazilian Institute for

Geography and Statistics; Brazil 2010, Brazil 2016b). Data on the residual native vegetation, river basins and phytogeographical regions were provided by Fundação SOS Mata Atlântica (SOS Mata Atlântica Foundation) and Instituto Nacionais de Pesquisas Espaciais (INPE; National Institute of Space Research; Atlântica & Inpe, 2013), as shown in Figs. 1 and 2. We gathered information on gender, age, clinical form, and proportion of CL patients, who achieved clinical cure. In the present study, we focused on municipalities with values of spatial density ≥0.010, which were considered to be areas with higher risk of transmission of the disease.

Geographical and statistical analysis

The geographical analysis was made using the SAR model (*simultaneous autoregressive model*; Bivand et al. 2008), that calculates the regression of values from several areas to model spatial dependence. Regression was carried out based on the coefficient of detection of CL (cases/100,000 inhabitants), as a function of the natural vegetation (km^2), percentage of natural vegetation, altitude, total number of cases and spatial density (cases per km^2). This means that the error is modeled based on its dependence on other errors. It is defined as:

$$e_i = \sum_{i=1}^{m} b_{ij} e_i + \varepsilon_i \ ,$$

where b_{ij} represents the values of spatial dependence between the areas; ε_i represents the residual error that is considered independent and identically distributed according to a normal with zero mean and constant variance.

All statistical analyses were carried out in the R environment (Team 2014).

RESULTS

Data on the native vegetation in the state of Paraná during 2012–2013 reveal that only 14% of the original vegetation cover remains (Fig. 2). Spatial regression of DC revealed statistical significance only for SD ($Z=22.1359$, $p<0.05$), while the values of other variables were: natural vegetation cover (km^2; $Z=1.0884$, $p>0.05$), percentage of natural vegetation cover ($Z=0.2303$, $p>0.05$), altitude ($Z=-0.8456$, $p>0.05$) and total number of cases ($Z=-1.3821$, $p>0.05$).

During 2012–2013, there were 513 reported cases of CL in the state of Paraná, across 85 municipalities (21.30%). Among these, six municipalities (7.06%) showed DC ≥71.0, 29 municipalities (34.12%) showed DC≥10.0<71.0, while 31 (36.47%) showed DC ≥2.5<10.0, and 19 (22.35%) showed DC <2.5. The municipalities with the highest number of cases were: Umuarama (64), Cianorte (43), Londrina (34), Bandeirantes (29) and Jussara (26). Together, these municipalities reported 38.20% of the CL cases in 2012 and 2013 (Table 1). The municipalities with the highest CD were: Jussara (191.83), Japurá (113.06), São Tomé (109.19), Tomazina (103.48), Adrianópolis (102.39) and São Jerônimo da Serra (78.73; Table 1), while 29 other municipalities had a detection coefficient ≥ 10.0, characterizing areas of high or very high transmission potential of CL. The SD analysis identified the prominent municipalities of Jussara (0.123), Japurá (0.121) and Bandeirantes (0.065; Table 1).

DISCUSSION

Despite the significance of CL based on the expressive number of cases reported annually and its occurrence in all Brazilian states, this disease continues to be neglected

(Monteiro et al. 2008, Brazil, 2016a). The extinction of the natural foci of CL due to the increase of human intervention did not occur, because this zoonosis has been identified in rural and urban zones in recent and past colonization (Lima et al. 2002, Brazil, 2016a).

Regression calculations show statistical significance ($p<0.05$) only for SD data in the municipalities of Cianorte, Corumbataí do Sul, Doutor Camargo, Japurá, Jussara, Lunardelli, Mandaguaçu, Maringá, Moreira Sale, Paraíso do Norte, São Tomé, Terra Boa, Tuneiras do Oeste and Umuarama, all of which are located in the CL generation hub, called the Ivaí-Pirapó hub by Monteiro et al. (2009). The semi-deciduous forest areas in these municipalities are represented by fragments of natural vegetation cover that are moderately or severely altered (Lima et al. 2002, Monteiro et al., 2008, Monteiro et al., 2009). No statistical significance ($p> 0.05$) was observed for the vegetation factor per km^2 and the percentage. Table 1 shows that Adrianópolis has 36% residual vegetation, with high DC (102.39), while the municipality of Jussara has only 10%, with high DC (191.83). Therefore, the *Leishmania* cycle is sustained, irrespective of the vegetation area in both the municipalities.

Though the municipalities of Bandeirantes, Pinhalão and Tomazina, included in the Cinzas-Laranjinha CL hub, have statistically significant ($p<0.05$) SD data, with statistically insignificant ($p>0.05$) natural vegetation cover (percentage and km^2), these municipalities are located in the hydrography corridors of areas covered with semi-deciduous forest remnants, where conditions suitable for the wild transmission cycle of *Leishmania* exist (Monteiro et al. 2009). The situation is similar in the Tibagi hub, where the municipalities of Londrina and São Gerônimo da Serra had statistically significant ($p<0.05$) SD data, with statistically insignificant ($p>0.05$) natural vegetation cover factor (km^2 and percentage).

In the Alto Ribeira hub, the municipalities of Adrianópolis and Cerro Azul had

statistically significant ($p < 0.05$) SD data, with statistically insignificant ($p > 0.05$) vegetation factor (per km^2 and percentage), where the hydrography corridors exist. These corridors are covered by the Atlantic forest, which allows the coexistence of *Leishmania*, vectors and reservoirs. The *Leishmania* cycle is sustained irrespective of the size of the natural vegetation covers of the municipalities.

Although municipalities with less than 0.010 have not been mentioned, it is important to note that, overall, all municipalities contribute to forming the Paraná-Paranapanema and Ribeira circuits.

In the areas of the state of Paraná where the native vegetation was replaced by coffee crops in the 1930s and 1950s, as well as by soy, maize, or pasture more recently (Lima et al. 2002, Monteiro et al. 2008, Monteiro et al., 2009), the infection of domestic and wild animals by *Leishmania* sp. (Lonardoni et al. 2006, Membrive et al. 2012, Truppel et al. 2014), the collection of sandflies in the residual forest areas (Luz et al. 2000, Teodoro et al. 2001, Cella et al. 2011, Massafera et al. 2005, Cruz et al. 2013) and recreational fishing activities (Lima et al. 2002) partly explain the higher density of CL cases in these areas. These studies *in loco* provide significant details about CL epidemiology. However, these are not detectable by the methodology used in this work.

The SINAN data used in geostatistics provide a broad coverage of the population of 399 municipalities in the state, facilitating the future continuity of the longitudinal study of CL epidemiology in Paraná, in order to assist with the health surveillance. However, the georeferencing instruments must be used with care, because in case of multifactorial diseases (CL, for instance), the enforcement of variables alone, and other computational models, without considering the local factors of exposed population and their habitat, and the interaction between them, can lead technicians, managers and researchers to incorrect findings (Lovelock 2010). Therefore, it is important to investigate

other local factors, such as the influence of the occupation of agrarian and urban spaces, migratory behavior, and vegetation cover, that may influence the risk factors for CL occurrence. These factors can determine spatial distribution, contributing to the occurrence of this disease in endemic areas (Monteiro et al. 2009).

CONCLUSION

Vegetation areas exert a strong influence on the occurrence of CL cases, because these are closely related to the ecology of vectors and reservoirs of the disease. So, concentration of LT cases occurs at the production hubs of this disease, where there is also a concentration of native vegetation, represented mainly by riparian forests

AUTHORS' CONTRIBUTIONS

HAM and UE conceived the study and wrote the manuscript. DFR contributed to statistical analyzes. All authors read and approved the final manuscript.

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FIGURE CAPTIONS

Fig. 1: Phytogeographical regions and river basins in the state of Paraná, Brazil (Maack, 1968)

Fig. 2: Residual vegetation cover in the state of Paraná, 2012–2013 (INPE, 2013)

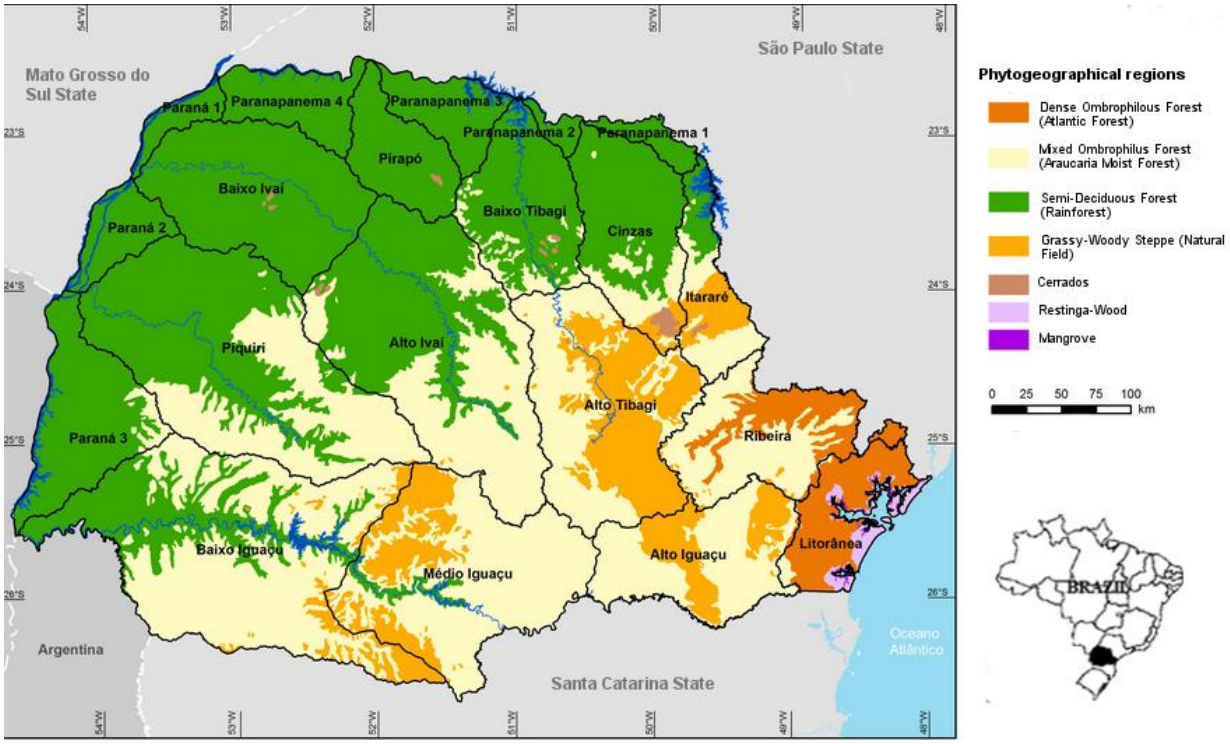

Fig. 1: Phytogeographical regions and river basins in the state of Paraná, Brazil (Maack, 1968)

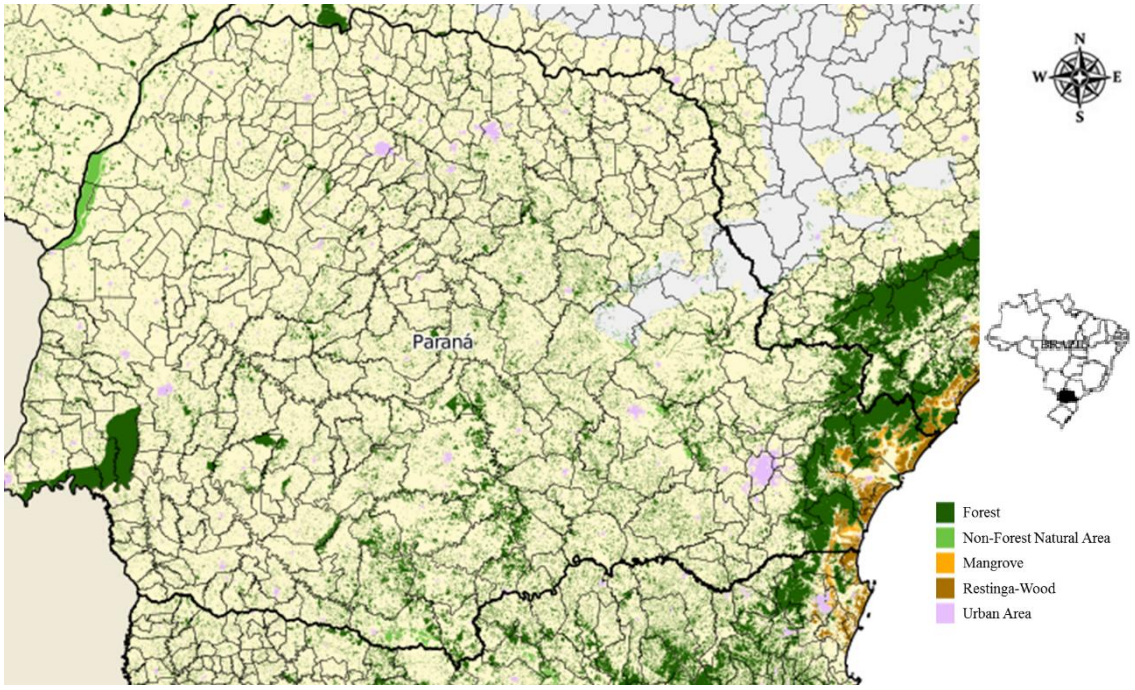

Fig. 2: Residual vegetation cover in the state of Paraná, 2012–2013 (INPE, 2013)

TABLE I

Number, spatial density and detection coefficient of cases of cutaneous leishmaniasis, natural vegetation covers in municipalities with spatial density ≥ 0.010, in the state of Paraná, Brazil 2012–2013

Municipality	NC	SD	DC	V	Municipality	NC	SD	DC	V
Adrianópolis	13	0.010	102.39	36	Londrina	34	0.021	3.23	11
Apucarana	8	0.014	3.19	10	Lunardelli	3	0.015	29.19	14
Arapongas	5	0.013	2.28	8	Mandaguaçu	3	0.010	7.25	4
Bandeirantes	29	0.065	44.79	3	Maringá	8	0.016	1.06	3
Cambira	2	0.012	13.40	8	Moreira Sales	4	0.011	15.82	2
Carlópolis	11	0.024	39.28	2	Paraíso do Norte	2	0.010	8.08	3
Cerro Azul	24	0.018	69.27	6	Pinhalão	6	0.027	47.53	6
Cianorte	43	0.053	29.21	9	São Carlos do Ivaí	4	0.018	30.56	2
Corumbataí do Sul	2	0.012	25.82	6	São Jerônimo da Serra	18	0.022	78.73	12
Doutor Camargo	7	0.059	59.02	2	São Tomé	12	0.055	109.19	12
Guaíra	9	0.016	14.24	12	Terra Boa	15	0.047	46.14	16
Itambaracá	9	0.043	66.19	2	Tomazina	18	0.030	103.48	4
Ivaiporã	5	0.012	7.76	3	Tuneiras do Oeste	7	0.010	39.92	15
Japurá	20	0.121	113.06	5	Umuarama	64	0.052	30.68	6
Jussara	26	0.123	191.83	10	**Total**	411	0.028	12.81	10

NC = Number of cases, SD = spatial density (cases per km^2); DC = detection coefficient (cases per 100,000 inhabitants); V = Natural Vegetation Covers (%)

CAPÍTULO III

CONCLUSÕES

Os casos de LT no estado do Paraná estão concentrados nas bacias do Ivaí-Pirapó, Tibagi, Cinzas-Laranjinha e Ribeira, onde é intense a exploração agrícola, especialmente nas três primeiras bacias. A análise estatística de dados georreferenciados para determiner a distribuição e as áreas de risco de LT pode ser utilizado como uma técnica de análise preliminar, pois a dimensão de qualquer agravo sem o conhecimento da situação da população e do ambiente pode resultar na visão virtual do problema e no planejamento de ações inadequadas para o seu controle.

As áreas de vegetação exercem forte influência na ocorrência de casos de LT, pois está intimamente relacionada à ecologia de vetores e reservatórios da doença, assim, a concentração de casos de LT ocorre nos pólos de produção desta doença, onde também há uma concentração de vegetação nativa, representada principalmente por florestas ciliares.

PERSPECTIVAS FUTURAS

O uso de técnicas com o uso de dados georreferenciados tem maior espaço na análise de dados nas áreas de saúde. Os achados encontrados durante a realização deste estudo mostram que o uso de dados dessas técnicas auxiliam no entendimento da distribuição de LT no estado do Paraná, servem como ferramenta de triagem para a análise adequada dos dados e direcionam o serviço público de saúde às ações de prevenção da doença. Há a necessidade de manter próximos estudos semelhantes a esse descrito para que se tenha um correto entendimento da epidemiologia da doença.

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Other elements	› Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. › Tables are inserted immediately after the first paragraph in which they are cited. › Supporting information files are uploaded separately.

Please refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

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Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author List

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

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Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.

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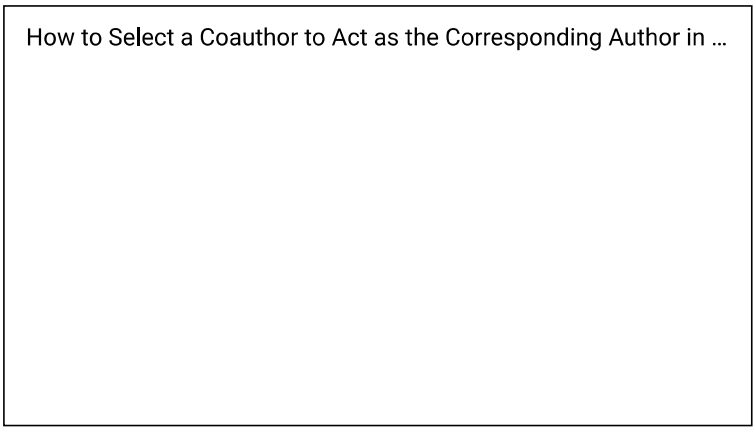

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If a manuscript is submitted on behalf of a consortium or group, include the consortium or group name in the author list, and include the full list of members in the Acknowledgments or in a supporting information file. Read the group authorship policy.

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Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

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- Not exceed 300 words

Abstracts should not include:

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- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

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The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information. Read the supporting information guidelines for formatting instructions. We recommend depositing **laboratory protocols** at protocols.io. Read detailed instructions for depositing and sharing your laboratory protocols.

Human or animal subjects and/or tissue or field sampling

Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. See the reporting guidelines for human research, clinical trials, animal research, and observational and field studies for more information.

Data

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For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to “data not shown.”

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Methods sections of manuscripts adding new taxon names to the literature must follow the reporting guidelines below for a new zoological taxon, botanical taxon, or fungal taxon.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

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Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

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Do not cite the following sources in the reference list:

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References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts or author summaries.

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Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

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Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (*Ailuropoda melanoleuca*). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005 *Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers.*
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Web sites or online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14.
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available from: arXiv:1403.3301v1. Cited 17 March 2014.
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html. Cited 17 March 2014.
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214.
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

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Example caption

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- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
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Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

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All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

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- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

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- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
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  - That the Institutional Review Board (IRB) approved use of oral consent
  - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
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- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

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Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

> *A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.*

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

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- Explain in the Methods the reason for failing to register before participant recruitment

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Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- Provide the registry name and number in the methods section of the manuscript
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's “Fig 1”

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

Animal research

We work in consultation with the *PLOS ONE* Animal Research Advisory Group to develop policies. Animal Research Advisory Group members may also be consulted on individual submissions.

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

If we note differences between an IACUC-approved protocol and the methods reported in a submitted manuscript, we may report these discrepancies to the relevant institution or committee.

Methods sections of manuscripts reporting results of animal research must include required ethics statements that specify:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s). Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why.
- Relevant details for efforts taken to ameliorate animal suffering

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Permit Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

The organism(s) studied should always be stated in the abstract. Where research may be confused as pertaining to clinical research, the animal model should also be stated in the title.

Where unregulated animals are used or ethics approval is not required, authors should make this clear in submitted articles and explain why ethical approval was not required. Relevant regulations that grant exemptions should be cited in full. It is the authors' responsibility to understand and comply with all relevant regulations.

We reserve the right to reject work that the editors believe has not been conducted to a high ethical standard, even if authors have obtained formal approval or approval is not required under local regulations.

We encourage authors to follow the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information. Please note that inclusion of a completed ARRIVE Checklist may be a formal requirement for publication at a later date.

Non-human primates

Manuscripts describing research involving non-human primates must include details of animal welfare, including information about housing, feeding, and environmental enrichment, and steps taken to minimize suffering, including use of anesthesia and method of sacrifice if appropriate, in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research* (PDF).

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional ethics consideration and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association (e.g., chloral hydrate, ether, chloroform) will not be considered at *PLOS ONE* unless authors also provide, at the time of initial submission, scientific and ethical justification for use in the specific study design, as well as confirmation of approval for specific use from their Institutional Animal Care and Use Committee (IACUC) or animal research ethics committee. Manuscripts reporting use of an anesthesia method(s) that is widely prohibited or of potential concern (chloral hydrate, ether, chloroform) should include a statement of scientific and ethical justification for use in the specific study design, as well as confirmation of approval for specific use from the authors' IACUC or animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

For additional guidance on appropriate euthanasia methods, authors may also refer to:

- Annex IV of the EU Directive 2010/EU/63 (PDF)
- CCAC Guidelines: on euthanasia of animals used in science (PDF)
- Report on the Second Newcastle Meeting on Laboratory Animal Euthanasia

Humane endpoints

For studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, *PLOS ONE* asks authors to report additional details related to the study design. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality. These studies may be subject to additional ethical considerations, and *PLOS ONE* may reject submissions if they lack sufficient reporting, appropriate justification for the study design, or adequate consideration of humane endpoints, regardless of study-specific institutional animal ethics committee approval.

Definition of a humane endpoint

A humane endpoint is an experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. These may include, for instance, body temperature or weight changes, tumor size or appearance, abnormal behaviors, pathological changes, ruffled fur, reduced mobility, body posture, or expression of specific body fluid markers. Please see the NC3Rs guidelines for more information.

Authors of these studies should report all of the following information in the Methods section:

1. Describe whether humane endpoints were used for all animals involved in the study

If humane endpoints were used, report the following:

- The specific criteria used to determine when animals should be euthanized
- Once animals reached endpoint criteria, the amount of time elapsed before euthanasia
- Whether any animals died before meeting criteria for euthanasia

If humane endpoints were not used, report the following:

- A scientific and ethical justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered but could not be used
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design

2. Include the following details of the study design and outcomes:

- The duration of the experiment
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals
- How frequently animal health and behavior were monitored
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions
- Any special training in animal care or handling provided for research staff

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

> *All necessary permits were obtained for the described study, which complied with all relevant regulations.*

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLOS ONE*'s data availability criterion.
- **Ethics.** *PLOS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

See our reporting guidelines for human subjects research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Manuscripts reporting results from blots (including Western blots) and electrophoretic gels should follow these guidelines:

- In accordance with our policy on image manipulation, the image should not be adjusted in any way that could affect the scientific information displayed, e.g. by modifying the background or contrast.
- All blots and gels that support results reported in the manuscript should be provided.
- Original uncropped and unadjusted blots and gels, including molecular size markers, should be provided in either the figures or the supplementary files.
- Lanes should not be overcropped around the bands; the image should show most or all of the blot or gel. Any non-specific bands should be shown and an explanation of their nature should be given.
- The image should include all relevant controls, and controls should be run on the same blot or gel as the samples.
- A figure panel should not include composite images of bands originating from different blots or gels. If the figure shows non-adjacent bands from the same blot or gel, this should be clearly denoted by vertical black lines and the figure legend should provide details of how the figure was made.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, or databases as the primary focus of the manuscript if they meet the following criteria:

Utility
The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation
Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability
Software should be open source, deposited in an appropriate archive, and conform to the Open Source Definition. Databases must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. Authors should provide a direct link to the deposited software or the database hosting site from within the paper.

Software submissions

Manuscripts describing software should provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifie (LSID), should be listed under the new species name, for example:

> ***Anochetus boltoni*** *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called “Nomenclatural Acts”:

> The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS [author to insert any additional repositories].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifie (LSID), should be listed under the new species name, for example:

> **Solanum aspersum** S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

> The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.
>
> In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifie (LSID), should be listed under the new species name, for example:

> ***Hymenogaster huthii.*** Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording (this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum):

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In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in PubMed Central and LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

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Running title: with up to 40 characters (letters and spaces)

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Abstracts: Provide an abstract of between 250- 300 words (100 words in case of short communications, technical notes, genome announcements or reviews). Abstracts of original articles should be structured into 5 sections as follows: BACKGROUND, OBJECTIVES, METHODS, FINDINGS and MAIN CONCLUSIONS, each section addressing respectively the problem, the aim of the study, the main methodological approach, the most important findings and the conclusions of the study.

Key words: 3-6 items must be provided. Terms from the Medical Subject Headings (Mesh) list of Index Medicus should be used.

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Results: should be a concise account of the new information discovered, with the least personal judgement. Do not repeat in text all the data in the tables and illustrations.

In case of describing New Species, should follow:

Name of the new species, authors (when it is the case), sp. nov., (Figs x-y)
[Ex: *An.* (*Nyssorhynchus*) *atacamensis* González and Sallum, sp. nov. (Figs 1-4)]

Previous reference to the new species (when it is the case)
[Ex: *An. pictipennis* of Rueda et al. (2008): 448.]

Diagnosis (or Description; all stages are described);

Type host (when it is the case);

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Discussion: should be limited to the significance of the new information and relate the new findings to existing knowledge. Only unavoidable citations should be included.

Acknowledgements: should be short and concise, and restricted to those absolutely necessary.

Author's contribution: state each author's contribution to the work.

REFERENCES

Must be accurate. Only citations that appear in the text should be referenced. Unpublished papers, unless accepted for publication, should not be cited. Work accepted for publication should be referred to as "in press" and a letter of acceptance of the journal must be provided. Unpublished data should only be cited in the text as "unpublished observations", and a letter of permission from the author must be provided. The references at the end of the paper should be arranged in alphabetic order according to the surname of the first author. CLICK HERE [+]

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Figures: presented in tiff format with a minimum of 300 dpi and photographs must be sharply focused, well contrasted, and if mounted onto a plate, the figures should be numbered consecutively with Arabic numbers. Magnification must be indicated by a line or bar in the figure, and referenced, if necessary in the caption (e.g., bar = 1 mm). Plates and line figures should either fit one column (8 cm) or the full width (16.5 cm) of the page and should be shorter than the page length to allow inclusion of the legend. Letters and numbers on figures should be of a legible size upon reduction or printing. A colour photograph illustrates the cover of each issue of the Journal and authors are invited to submit illustrations with legends from their manuscript for consideration for the cover.

Tables: should supplement, not duplicate, the text and should be numbered with Roman numerals. A short descriptive title should appear above each table, with any explanations or footnotes (identified with a, b, c, etc.) below.

Review: Papers in “review” format are accepted only by means of invitations made by the editor or associated editors.

Technical Notes: Technical Notes should communicate rapidly single novel techniques or original technical advances. The entire note should occupy no more than three printed pages including figures and/or tables (it means around 10 double-spaced typed Word file maximum). The text must not be not divided into sections. Therefore, the state of art must be very briefly presented; results must be rapidly presented and discussed at a time. Complementary tables and figures may be published as supplementary data. References must be limited to few essential ones and cited at the end of the note, using the same format as in full papers. A brief summary and three key words must be provided.

Short communications: should communicate rapidly single results or techniques. They should occupy no more than three printed pages including figures and/or tables. They should not contain excessive references. References should be cited at the end of the paper using the same format as in full papers. A brief summary and three key words must be provided.

Genome Announcement and Highlights: this section is dedicated to publish new genome information from eukaryote parasites, virus, bacteria and their respective vectors. Authors who wants a fast peer review and publication cycle for their research results covering new genome sequences, re-sequencing and comparative genome analysis as well as the expression pattern of genomes are invited to submitted papers under the short communication format.

Alternative format: manuscripts may be submitted following the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" produced by the International Committee of Medical Journal Editors also known as the Vancouver Style. In this case, authors should follow the guidelines in the fifth edition (Annals of Internal Medicine 1997; 126: 36-47, or at the website http://www.acponline.org/journals/resource/unifreqr/htm) and will be responsible for modifying the manuscript where it differs from the instructions given here, if the manuscript is accepted for publication. Authors should also follow the Uniform Requirements for any guidelines that are omitted in these Instructions.

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