

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL – UFRGS

**Geo-helmintíases a partir da perspectiva de Saúde Única: atualização e achados em amostras de solo da cidade de Porto Alegre**

*Soil-transmitted helminths from the One Health perspective: an update and findings in soil samples from Porto Alegre city*

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“O que mais me emociona é que o que não vejo contudo existe. Porque então tenho aos meus pés todo um mundo desconhecido que existe pleno e cheio de rica saliva.”<sup>1</sup>

Clarice Lispector

<sup>1</sup> Texto extraído do livro “Água Viva” (Lispector, C, 1973, p. 88)



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## RESUMO

Os geo-helminhos *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos (*Ancylostoma duodenale* e *Necator americanus*) são um grupo de parasitas intestinais que apresentam parte obrigatória de seu desenvolvimento no solo. Existem diversos fatores que podem influenciar sua incidência nas cidades, como questões de habitação, saneamento ambiental e acesso aos serviços de saúde. A infecção por geo-helminhos é associada com deficiências nutricionais, problemas gastrointestinais e déficits no desenvolvimento infantil e gera grande efeito sobre os índices de desenvolvimento humano em países de baixa e média renda. Considerando este cenário, o objetivo desse trabalho foi analisar e atualizar as informações sobre a prevalência desses parasitas em amostras de solos coletadas em diferentes regiões de Porto Alegre, Rio Grande do Sul. O primeiro capítulo desta dissertação discute aspectos da cidade de Porto Alegre que podem se relacionar com a circulação de parasitas na população. O segundo capítulo discute e revisa, sob a perspectiva de Saúde Única, os estudos realizados em amostras humanas, animais e ambientais sobre a distribuição das geo-helminthíases no Rio Grande do Sul. No terceiro capítulo são abordadas as relações da infecção de geo-helminhos e a deficiência de ferro em humanos. No quarto capítulo são apresentados dados do estudo piloto realizado no Campus do Vale, onde foram coletadas e analisadas 40 amostras de solo para a presença de ovos e larvas de geo-helminhos. O quinto capítulo traz os resultados das coletas de solo em parques e praças públicas nas oito regiões de planejamento de Porto Alegre, nas quais foram feitas análises de microscopia e moleculares para a detecção dos geo-helminhos *Toxoplasma gondii* e *Toxocara canis* e *T. cati*. Por fim, o capítulo VI discute os geo-helminhos como integrantes das doenças tropicais negligenciadas e os fatores que influenciam a prevalência dessas doenças nas cidades. Em conjunto, esta dissertação aborda, de forma bastante ampla, a prevalência e infecção por geo-helminhos sob a perspectiva de Saúde Única, com enfoque na cidade de Porto Alegre.

**Palavras-chave:** geo-helminhos, parasitas, Porto Alegre, saúde ambiental, solo, Saúde Única

## ABSTRACT

The soil-transmitted helminths (STH) *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*) are a group of intestinal parasites that have an obligatory part of their development cycle in the soil. There are several factors that can influence its incidence in cities, such as housing issues, environmental sanitation, and access to health services. Infection by STH is associated with nutritional deficiencies, gastrointestinal problems and deficits in child development, and has an important effect on human development indices in low- and middle-income countries. Considering this scenario, the objective of this work was to analyze and update information on the prevalence of these parasites in soil samples collected in different regions of Porto Alegre, Rio Grande do Sul. The first chapter of this dissertation discusses aspects of the city of Porto Alegre that can be related to the dispersion of parasites in the population. The second chapter discusses and reviews, from the One Health perspective, the studies carried out in human, animal and environmental samples on the distribution of STH in Rio Grande do Sul. The third chapter discusses the relationship between STH infection and iron deficiency in humans. The fourth chapter presents the pilot study carried out at *Campus do Vale*, where 40 soil samples were collected and analyzed for the presence of eggs and larvae of STH. The fifth chapter brings the results of soil collection from parks and public squares in the eight planning regions of Porto Alegre, in which microscopy and molecular analyzes were carried out for the detection of STH, *Toxoplasma gondii* and *Toxocara canis* and *T. cati*. Finally, chapter VI discusses STH as part of neglected tropical diseases and the factors that influence the prevalence of these diseases in cities. Together, this dissertation approaches, in a broad way, the prevalence and infection by STH from the One Health, perspective focusing on the Porto Alegre city.

**Keywords:** environmental health, parasites, Porto Alegre, soil, soil-transmitted helminths, One Health

## APRESENTAÇÃO

Esta dissertação reúne trabalhos que analisaram a distribuição e biologia dos geohelminhos e outros parasitas no contexto urbanístico, ambiental e social de Porto Alegre, Rio Grande do Sul, utilizando como base a perspectiva de Saúde Única (*One Health*). O **Capítulo I** introduz alguns assuntos que influenciam as características de distribuição e transmissão de parasitas e apresenta questões do desenvolvimento urbano e do saneamento básico da cidade de Porto Alegre.

O **Capítulo II** traz uma revisão das informações sobre geohelminhos no Rio Grande do Sul, utilizando estudos realizados com amostras humanas, animais e ambientais. Além disso, discute os aspectos biológicos de geohelminhos e aborda os potenciais impactos das alterações ambientais observadas no Rio Grande do Sul sobre a ocorrência das geohelmintíases. O **Capítulo III** discute o papel da infecção por geohelminhos na deficiência de ferro e explora as conexões clássicas e negligenciadas entre infecção por geohelminhos, geofagia, resposta imune e deficiência de ferro. O **Capítulo IV** apresenta o estudo piloto que analisa, através de microscopia, a presença de geohelminhos no solo de diferentes pontos distribuídos pelo Campus do Vale (Universidade Federal do Rio Grande do Sul). Em seguida, no **Capítulo V**, são apresentados resultados de análises microscópicas e moleculares de coletas de amostras de solo realizadas em diferentes locais de Porto Alegre, juntamente com uma discussão sobre a distribuição dos parasitas na cidade.

Por fim, o **Capítulo VI** apresenta uma discussão sobre os temas abordados na dissertação, as conclusões finais e as perspectivas deste trabalho. O **Anexo A** contém a produção científica complementar realizada pela autora durante o período do mestrado.

## CAPÍTULO I

### INTRODUÇÃO

#### *Parasitas, ecologia e ecossistemas: uma questão de Saúde Única*

A infecção parasitária apresenta uma dinâmica complexa de transmissão entre os hospedeiros, níveis tróficos e o ambiente (Jenkins et al., 2015). Os parasitas são organismos onipresentes que podem influenciar o comportamento, crescimento, natalidade e mortalidade de seu hospedeiro (Marcogliese, 2004). Considerando os diversos ecossistemas em que estão inseridos, os parasitas podem modelar a dinâmica e a estrutura da comunidade de seus hospedeiros, alterar a competição interespecífica e influenciar o fluxo de energia, desempenhando papel significativo como condutores da biodiversidade (Hudson et al., 2006). Por exemplo, após a demonstração inicial de que infecções latentes por *Toxoplasma gondii* podem alterar o comportamento em roedores, tornando-os mais suscetíveis à predação por felídeos (Webster, 2007), observaram-se mudanças comportamentais de risco e de impulsividade associadas à infecção em diversos hospedeiros intermediários deste parasita, incluindo em seres humanos (Flegr, 2007; Meyer et al., 2022). Além disso, a infecção por *T. gondii* pode ocasionar elevação nas taxas de morbidade e mortalidade, principalmente em indivíduos imunossuprimidos, gestantes e em populações de animais que não co-evoluíram com felídeos (Hollings et al., 2013). A combinação de efeitos diretos e indiretos resultantes da infecção pode alterar a dinâmica das interações tróficas e das interações interespecíficas, e, portanto, o fluxo de energia do ecossistema (Marcogliese, 2004; Hudson et al., 2006).

Embora não haja uma forma direta de mensurar a “saúde do ecossistema”, pode-se utilizar metaforicamente esse conceito para avaliar o estado atual comparando-se a um estado ambiental desejado (Marcogliese, 2005). De forma prática, esse conceito é utilizado para transmitir preocupações ambientais aos diversos públicos. Ecossistemas saudáveis podem ser definidos como aqueles ecossistemas que mantêm vigor, organização e resiliência, apresentando uma grande variedade de parasitas (Hudson et al., 2006). Embora inicialmente essa afirmativa pareça contraditória e indesejada do ponto de vista da saúde pública, a

presença de parasitas generalistas e especializados em diferentes níveis tróficos contribui para estruturar e estabilizar as populações, fortalecendo conexões multiespécies, aumentando a resiliência e resistência das populações infectadas (Rapport et al., 1998; Costanza e Mageau, 1999; Hudson et al., 2006).

Alguns helmintos, por exemplo, desenvolveram métodos de infecção bastante complexos, com ciclos de vida que envolvem vertebrados e invertebrados e dependem de diferentes condições ambientais. Perturbações na biodiversidade e na dinâmica da teia alimentar afetam diretamente a transmissão do parasita, alterando a composição e abundância parasítica (Marcogliese, 2005). É o caso do geo-helminto *Strongyloides stercoralis*, que possui um ciclo de vida complexo que pode incluir diferentes hospedeiros (sendo humanos os hospedeiros definitivos) no ciclo parasítico e estágios de desenvolvimento direto ou indireto durante o ciclo de vida livre. O desenvolvimento da larva se dá obrigatoriamente no solo e depende de diversos fatores ambientais que afetam sua epidemiologia e distribuição, ocorrendo majoritariamente em regiões tropicais (Hernández-Castro, 2014; CDC, 2019).

Os parasitas podem ajudar a elucidar informações ecológicas importantes sobre seu hospedeiro e também sobre o ambiente em que estão inseridos (Marcogliese e Cone, 1997). Uma metanálise realizada por Vidal-Martínez et al. (2009) encontrou associação significativa entre alterações ambientais (causadas por poluentes e estressores ambientais) e o número populacional, a fisiologia e a composição química de parasitas (Vidal-Martínez et al, 2009). Dessa forma, parasitas podem servir como bioindicadores de perturbações ambientais induzidas por fatores antropogênicos e ambientais, como poluição, alterações climáticas e exposição a tóxicos que podem alterar a composição de grandes ecossistemas (Marcogliese, 2005; Vidal-Martínez e Wunderlich, 2017).

A transmissão de parasitas dentro dos ecossistemas pode ocorrer de diferentes formas e depende de fatores humanos, animais e ambientais. O conceito de Saúde Única (*One Health*) determina que deve-se analisar de forma conjunta os diferentes cenários em que diversos patógenos (incluindo parasitas) atuam, demonstrando a interdependência entre a saúde humana, animal e ambiental (Ellwanger e Chies, 2022). O ambiente participa da rota de diversas doenças parasitárias e pode atuar como um reservatório, abrigando parasitas que, por

sua vez, podem contaminar humanos e outros animais. Além disso, humanos podem transmitir parasitas, direta ou indiretamente, aos outros animais (zooantroponoses). Da mesma forma, animais podem transmitir parasitas patogênicos para os humanos (zoonoses ou antropozoonoses) (Ziliotto et al., 2022).

Nas cidades, diversos fatores podem desencadear desequilíbrios do ecossistema que resultam em surtos e epidemias de diversos patógenos (Almeida et al., 2020). Por exemplo, a falta de saneamento ambiental eficiente é um dos principais geradores de transmissão de geohelminhos (da Silva et al., 2019). Em Salvador, Bahia, uma intervenção de saneamento por toda a cidade que promoveu um crescimento de cobertura de 26% para 80% de casas com tratamento adequado de esgoto diminuiu significativamente a incidência e prevalência de geohelminhos na região (Mascarini-Serra et al., 2010). Além disso, no contexto urbano, a prevalência e transmissão de parasitas é influenciada por diversas questões sociais, como moradia adequada, questões urbanísticas de acesso a serviços de saúde e infraestrutura deficitária na distribuição de água potável e tratamento adequado de esgoto (Almeida et al., 2020; Ziliotto et al., 2022). Nesse sentido, a extensão do conceito de Saúde Única para uma Saúde Única Urbana (*Urban One Health*) se faz necessária para o estudo e controle das doenças infecciosas e parasitárias nas cidades (Ellwanger et al., 2022a).

#### *Porto Alegre: fatores urbanísticos e populacionais*

O desenvolvimento urbano define a ecologia humana moderna (McMichel, 2000). A sustentabilidade no sistema urbano pode ser relacionada com a forma de ocupação do território; a disponibilidade de insumos e destino e tratamento de esgoto e lixo; a mobilidade da população dentro do sistema urbano; e o atendimento das necessidades da população em relação à moradia, serviços sociais e qualidade dos espaços públicos (Grostein, 2001). De forma complementar, os problemas ambientais urbanos se relacionam aos diferentes processos que envolvem decisões socioeconômicas, culturais e políticas em torno da formação da cidade e que acabam por influenciar na configuração do espaço, expondo as relações interclasses (Grostein, 2001). A ecologia urbana estuda a relação de organismos entre si e sua relação com ambientes de alta densidade de desenvolvimento residencial e comercial. Esses ambientes são influenciados pela presença humana de diversas formas. À vista disso, as ciências humanas,

exatas e da natureza contribuem conjuntamente para o estudo da ecologia urbana (Douglas e James, 2015).

A urbanização brasileira teve seu início em meados do século XX, sendo impulsionada pelo processo de industrialização que ocorreu entre 1960 e 1970 e resultou no aumento populacional das áreas urbanas (Ferreira et al., 2016). Em 1970, o Rio Grande do Sul já apresentava maior parte da sua população (53,6%) residente em áreas urbanas em relação às áreas rurais (Ferreira et al., 2016). Por um lado, a formação de aglomerações urbanas e cidades representa um fator estratégico para a concentração de serviços sociais e desenvolvimento econômico, além de contribuir para o desenvolvimento de diversos avanços sociais, culturais, científicos e técnicos através da facilitação do intercâmbio de informações (Tartaruga, 2016; Azambuja et al., 2016). Por outro lado, a população residente que convive com a pobreza e falta de infraestrutura está exposta ao desenvolvimento de diversas doenças associadas com desigualdades sociais e problemas urbanos, como doenças infecciosas relacionadas à poluição ambiental, doenças crônicas cardiometabólicas, transtornos mentais, exposição a drogas e a diversas formas de violência (Azambuja et al., 2016). Essa dicotomia em relação à saúde no ambiente urbano pode ser chamada de “paradoxo da ecologia urbana de doenças” (*urban disease ecology paradox*) (Ellwanger et al., 2022a). Dessa forma, a desigualdade na infraestrutura e no acesso a serviços resulta em desigualdades sociais (Azambuja et al., 2016).

De forma muito similar a outras capitais, Porto Alegre se desenvolveu em metrópole de forma conjunta com a industrialização da região. A rápida transformação fez com que, entre 1950 e 1980, a cidade apresentasse um crescimento populacional exponencial (Figura 1), gerando uma fragmentação do espaço que causou exclusão social e territorial, impactando a provisão de moradias até os dias atuais (Lahorgue et al., 2022; Nugem et al., 2021). As ocupações irregulares (ou aglomerados subnormais) se caracterizam por serem áreas sem infraestrutura básica, ausência de saneamento ambiental, escolas e serviços de saúde, similar à constituição de periferias (Heidrich et al., 2016; IBGE, 2019).



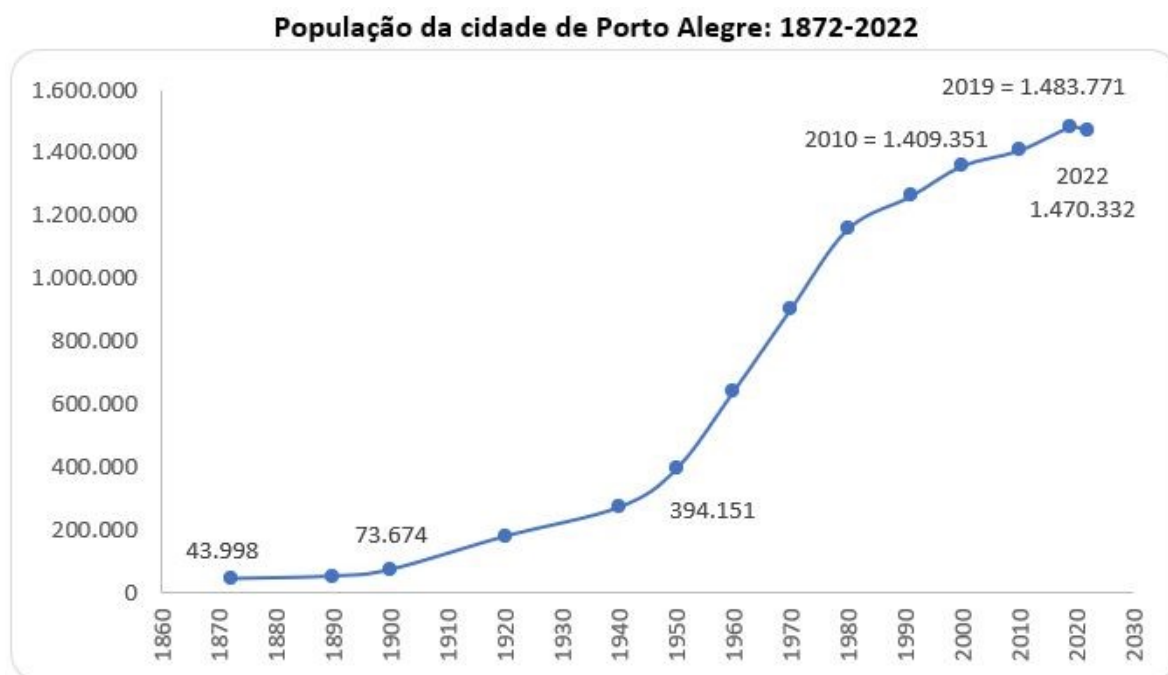


Figura 1: censo demográfico da população da cidade de Porto Alegre. Dados do IBGE.  
 Fonte: site Ecodebate: <https://www.ecodebate.com.br/2022/07/07/porto-alegre-tres-anos-seguidos-de-decrescimento-demografico/>

Geograficamente, as áreas de ocupações irregulares podem estar incorporadas à área central da cidade como lotes urbanos e áreas públicas que representam um maior acesso à infraestrutura urbana, porém geralmente apresentam riscos à vida - estando mais suscetíveis a acidentes naturais e incêndios - e não possuem saneamento ambiental adequado, englobando, por exemplo, margens de córregos e escoadouros de esgotos (Heidrich et al., 2016; Nagem et al., 2021). A Figura 2 apresenta a localização dos aglomerados subnormais em Porto Alegre nos anos de 2010 e 2019 (Lahorgue et al., 2022). Nela, é possível observar a relação entre as ocupações irregulares e a localização de morros e locais inundáveis da cidade, que são locais subjulgados pelo mercado imobiliário ou vedados pela legislação (Lahorgue et al., 2022).

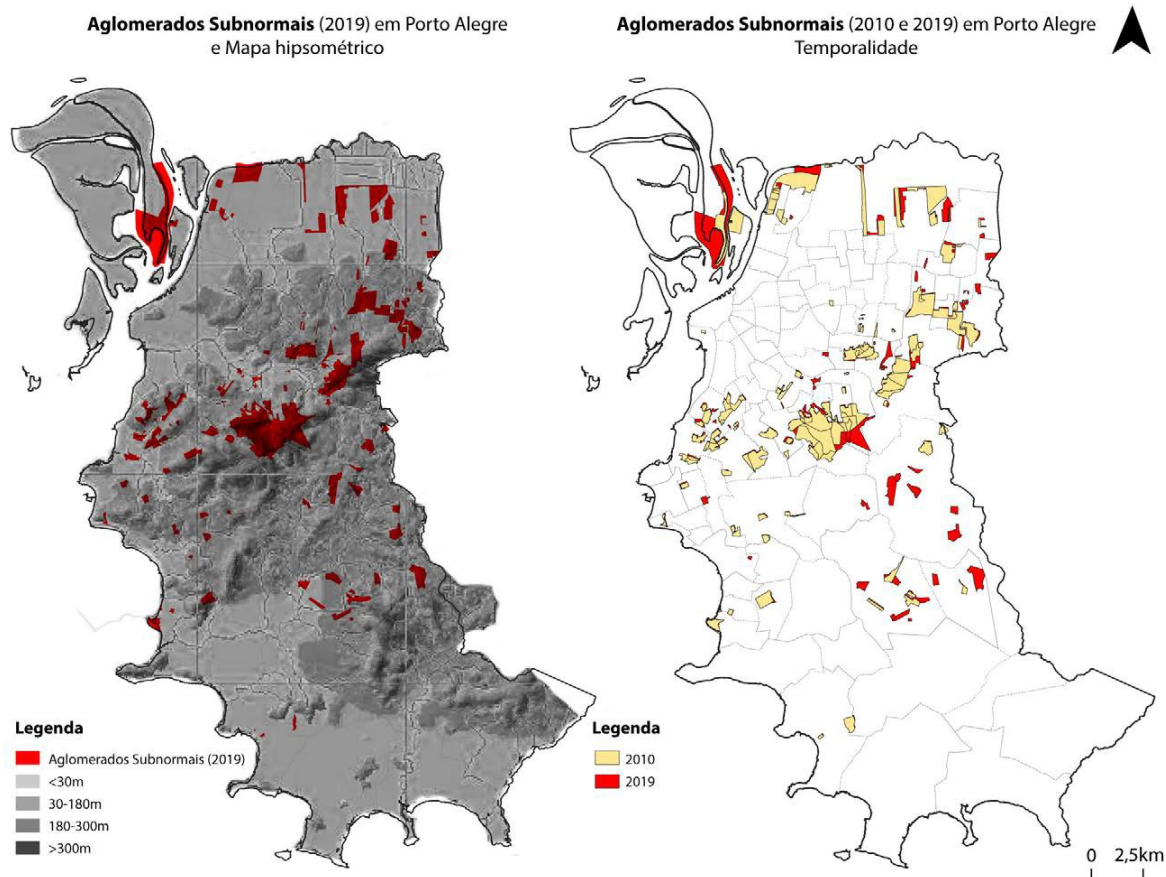


Figura 2: Aglomerados subnormais (2010 e 2019) e mapa hipsométrico. Fonte: retirada e modificada de Lahorgue et al. (2022).

### *Saneamento ambiental e saúde da população em Porto Alegre*

Uma das formas de garantir melhora na igualdade social e diminuição da degradação do ambiente é o acesso universal ao saneamento ambiental e condições próprias de moradia (McMichel, 2000; Carvalho e Adolfo, 2012). O saneamento ambiental é definido por ações direcionadas à sociedade que incluem acesso ao abastecimento de água potável, coleta e disposição sanitária de resíduos sólidos, líquidos e gasosos, disciplina sanitária de uso do solo e ocupação da terra e obras relacionadas a proteção e melhoria das condições de vida da população e meio ambiente, além do controle ambiental de vetores e reservatórios de doenças transmissíveis, para que a população urbana e rural mantenha condições de vida dignas (Nugem et al., 2021; Brasil, 2005). O novo marco regulatório de saneamento brasileiro,

sancionado em julho de 2020 através da lei nº 14.026/2020, postulou como meta, até dezembro de 2033, o acesso de 99% da população brasileira à água potável e 90% da população à coleta e tratamento de esgoto (ANA, 2020).

Desde a aprovação da lei que sancionou o Plano Diretor de Desenvolvimento Urbano Ambiental do Município de Porto Alegre (PDDUA) (Lei Complementar Municipal nº 434/1999) se propunham, como metas de planejamento urbano da cidade, estratégias para lidar com a poluição e degradação ambiental com a intenção de alcançar melhorias nas condições sanitárias e a valorização do patrimônio ambiental (SPM, 2010). O último plano de saneamento básico de Porto Alegre (PMSB) (DMAE, 2015) contempla a prestação de serviços públicos de natureza essencial (abastecimento de água, esgotamento sanitário, manejo de águas pluviais e resíduos sólidos) e apresenta a universalização do serviço de coleta e tratamento de esgoto como uma das metas a ser atingida até 2035 com reflexos diretos na melhoria da qualidade de vida da população, compreendendo que a saúde humana e ambiental estão interconectadas (DMAE, 2015).

Porém, o período entre 2016 e 2018 apresentou índices de piora na rede de tratamento de esgoto. Em 2019 e 2020, ainda que a cidade tenha apresentado melhoras nas métricas, o desempenho continuou pior quando comparado com 2015, mesmo com a inauguração das obras do Pisa (Projeto Integrado Socioambiental), que foram entregues em 2014 e pretendiam alcançar o tratamento de 80% do esgoto coletado na cidade de Porto Alegre (Dal Maso, 2016; Rollsing, 2022). A Estação de Tratamento de Esgoto (ETE) Serraria foi a principal obra entregue durante o Pisa, a fim de que o esgoto bruto da região da zona Sul de Porto Alegre deixasse de ser lançado no lago Guaíba, o principal manancial de abastecimento de água para consumo humano da cidade (de Souza, 2019).

Apesar de Porto Alegre já ter atingido a meta de universalização de acesso à água potável pela população residente, dados do Sistema Nacional de Informações Sobre Saneamento (SNIS) demonstram uma estagnação nos índices de tratamento e coleta de esgoto, como demonstrado na Figura 3. Segundo reportagem do jornal Zero Hora (Rollsing, 2022), isso se deve a falhas na correção da ligação de imóveis com a rede de esgoto da região e a falta de ampliação das obras. Mesmo com grande capacidade de tratamento, a falta de ampliação e

manutenção dos serviços faz com que dejetos brutos deixem de ser coletados e sejam despejados diretamente no Guaíba (Rollsing, 2022). No Ranking de Saneamento Básico do Instituto Trata Brasil, que utiliza dados oficiais do SNIS para avaliar a evolução dos indicadores de água, esgotos, investimentos e perdas de água nas maiores cidades do Brasil, Porto Alegre segue perdendo posições. Em 2019, a capital estava na 38º posição (SNIS 2020), em 2020 estava em 40º (SNIS, 2020), em 2021, desceu para a 42º (SNIS, 2022), atingindo, em 2022, a 43º posição (SNIS 2022).

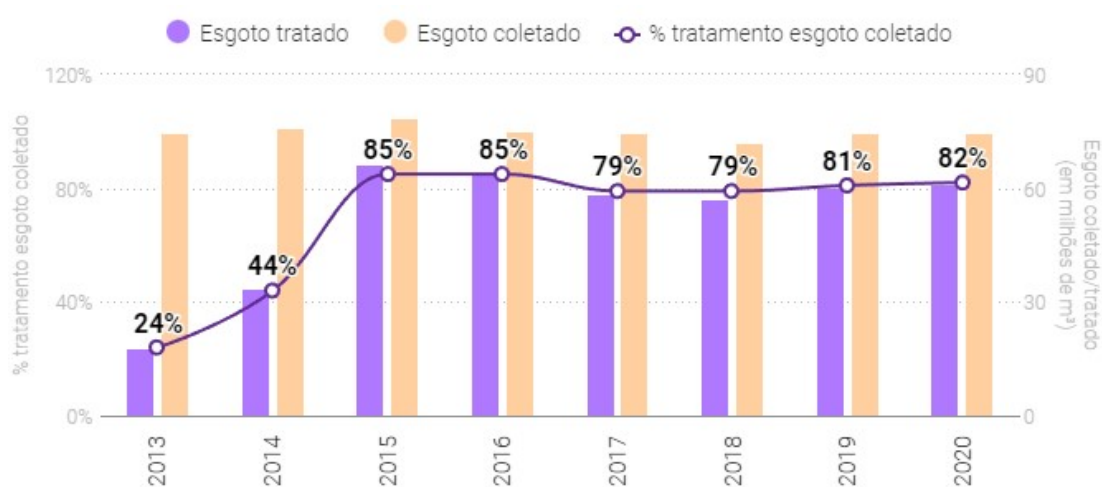


Figura 3: gráfico de indicadores de esgoto tratado e coletado, segundo dados retirados de SNIS. Fonte: Retirado de Rollsing (2022).

### *Saúde ambiental, desigualdades sociais e doenças parasitárias em Porto Alegre*

Os problemas acerca da proliferação de doenças infectocontagiosas estão diretamente ligados ao desenvolvimento e a desigualdades de acesso e moradia das cidades (Almeida et al., 2020). A ocorrência de diversas doenças parasitárias negligenciadas está associada a fatores socioeconômicos que variam entre populações, como baixo nível educacional, analfabetismo parental, pais desempregados, baixa renda familiar, entre outros (Nematian et al., 2004; Ulukanligil e Seyrek, 2004; Ngui et al., 2012). Em Porto Alegre, a prevalência da infecção por *Toxoplasma gondii* em idosos foi associada estatisticamente com baixo nível de estudo e

menor renda pessoal (Engroff et al., 2014). Além disso, as áreas da cidade que apresentaram maior incidência de infecção (região Sul/Centro Sul e região Noroeste/Humaitá/Navegantes/Ilhas) possuem condições habitacionais e de estrutura pública piores que as demais regiões do município de Porto Alegre (Engroff et al., 2014).

Em Porto Alegre, uma avaliação ambiental de parques e praças públicas realizada entre o período de 2001 e 2004 revelou uma contaminação de 100% dos locais, onde foram encontrados ovos de *Ascaris* spp. (10,2%), ovos de *Trichuris* spp. (4,4%) e ovos de *Toxocara* (4,2%), além de larvas de helmintos (18,0%) (Vargas et al., 2013), indicando uma infestação ambiental preocupante em termos de saúde pública. Ainda, considerando as demais doenças tropicais negligenciadas, Porto Alegre enfrenta casos de *Leishmania*, uma zoonose causada pelo protozoário do gênero *Leishmania* e transmitida pelo flebotomíneo vetor popularmente conhecido como “mosquito-palha” (Harhay et al., 2011; Prefeitura de Porto Alegre, 2022). Leishmaniose é uma doença crônica que apresenta alta letalidade, principalmente em indivíduos vulneráveis. No Brasil, o cão é considerado o principal reservatório de *Leishmania* em áreas urbanas (Coura-Vital et al., 2011). A primeira notificação na capital ocorreu em 2016 e desde então, 113 casos suspeitos foram notificados (Prefeitura de Porto Alegre, 2022). Destes, 20 casos foram autóctones, 19 dos quais ocorreram em moradores de ocupações irregulares em regiões próximas à mata e sem acesso a saneamento básico (Prefeitura de Porto Alegre, 2022; Mahmud et al., 2019). Além desses fatores que influenciam a incidência da Leishmaniose, em zonas sub-tropicais as mudanças climáticas e de temperatura apresentam uma associação com a quantidade de vetores presentes no ambiente (Rêgo et al., 2020).

Porto Alegre apresentou, em 2022, um aumento de casos de dengue. A dengue é uma doença infecciosa causada por arbovírus e transmitida pelo mosquito do gênero *Aedes* (Brasil, 2002). A infecção pode causar febre alta e dores pelo corpo. O aparecimento de manchas vermelhas na pele e dores abdominais intensas indica casos graves de dengue hemorrágica, que podem ser fatais (Brasil, 2002). O aumento de casos ocorridos em Porto Alegre acende um alerta para um padrão de aumento que vem ocorrendo ao longo dos últimos 50 anos (COE/UFCSPA, 2022). As mudanças antrópicas no uso de terras como urbanização não planejada e desmatamento, fatores relacionados a alterações climáticas e aquecimento global

afetam a reprodução e dispersão dos vetores (Ellwager et al., 2022b; COE/UFCSPA, 2022) e podem estar influenciando diretamente o aumento de casos na cidade. Efetivamente, altas temperaturas, variabilidades climáticas, cobertura vegetal e uso antrópico já foram relacionados anteriormente com o aumento de números de casos em Porto Alegre (Collischonn et al. 2019; Penso-Campos et al., 2018).

Porto Alegre está inserida em uma região de transição entre os biomas Mata Atlântica e Pampa, apresentando um mosaico de características geomorfológicas e biológicas de ambos os biomas. Os biomas naturais presentes no Rio Grande do Sul sofrem grandes perdas anuais devido ao aumento na extensão de cultivos de monoculturas, pastagens e agricultura (MapBiomias, 2021). A monocultura de soja tomou o lugar da pecuária extensiva em pastagens naturais nos últimos anos, aumentando as áreas plantadas em 188,5% entre 2000 e 2015 (Krob et al., 2021). Em 2021, o bioma Pampa apresentou um aumento da perda de vegetação de 92% comparado ao ano anterior, ameaçando sua biodiversidade e aumentando o risco de disseminação de zoonoses (MapBiomias 2021, Ellwanger et al., 2022c). Considerando que as mudanças climáticas e ambientais afetam diretamente os vetores responsáveis por diversas doenças tropicais negligenciadas (DTN), incluindo geo-helmintos, e que cada região apresenta diferentes características socioambientais (Furie e Balbus, 2012; Valero-Bernal e Tanner, 2008), é importante considerarmos como as mudanças ambientais e urbanas vêm afetando a disseminação dessas doenças.

O saneamento ambiental é um fator determinante para a saúde populacional e se relaciona de diferentes maneiras com a infecção e distribuição de parasitas na cidade, conforme já discutido anteriormente. A falta de saneamento básico adequado aumenta a transmissão de doenças infecto-parasitárias que têm o ambiente como potencial determinante, chamadas de doenças relacionadas ao saneamento ambiental inadequado (DRSAI) (Siqueira et al., 2017). As DRSAI causaram, entre 2010 e 2014, 13.929 internações no município de Porto Alegre (Siqueira et al., 2017). Estabelecer relações entre meio ambiente e saúde é importante para a prevenção da incidência e proliferação de diversas doenças infecto-contagiosas, que estão presentes no processo de urbanização (Almeida et al., 2020). Para isso, é fundamental integrar conhecimentos da área da saúde com ações de política social e educação ambiental,

buscando a mobilização da sociedade, habitação adequada para todos, saneamento ambiental universal e saúde ambiental. Neste contexto, Porto Alegre se mostra como uma cidade ideal como modelo de estudo para essas questões.

## OBJETIVOS

### Objetivo geral

Investigar, através de técnicas de parasitologia clássica e de biologia molecular, a presença de parasitas em amostras de solo de diferentes regiões da cidade de Porto Alegre, relacionando os resultados encontrados com fatores socioeconômicos, sanitários e ecológicos de cada região amostrada, sob a perspectiva de Saúde Única.

### Objetivos específicos

I) Investigar a presença de ovos/larvas de geo-helminthos em amostras de solo através de técnicas de microscopia;

II) Analisar a presença do DNA de *Toxoplasma gondii*, *Toxocara canis* e *Toxocara cati* em amostras de solo;

III) Comparar a presença/ausência de parasitas em amostras de solo entre as diferentes regiões estudadas;

IV) Relacionar a presença de parasitas em amostras de solo de diferentes regiões da cidade de Porto Alegre com fatores sociais, sanitários e ecológicos de cada local amostrado.



## CAPÍTULO II

### *Geo-helminthiases no Rio Grande do Sul*

Este capítulo apresenta um trabalho publicado na revista Bio Diverso que revisa, sob a perspectiva de Saúde Única, estudos que analisaram a presença de geo-helminthos em amostras humanas, animais e ambientais no Rio Grande do Sul.

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## REVISÃO E SÍNTESE

# Geo-helmintíases no Rio Grande do Sul: uma análise a partir da perspectiva de Saúde Única

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**Resumo:** Os helmintos intestinais *Ascaris lumbricoides*, *Trichuris trichiura* e os ancilostomídeos *Ancylostoma duodenale* e *Necator americanus* apresentam importante relevância em termos de saúde pública no Brasil, sendo associados com deficiências nutricionais, problemas gastrointestinais e déficits no desenvolvimento infantil. Essas espécies são conhecidas conjuntamente como geo-helmintos, pois o solo (*geo*) exerce papel importante na transmissão e no desenvolvimento desses parasitas. A contaminação ambiental por ovos e larvas de geo-helmintos é particularmente comum em áreas com problemas de distribuição de água potável e no tratamento de esgoto. Humanos infectados por *A. lumbricoides*, *T. trichiura* e ancilostomídeos podem liberar uma grande quantidade de ovos dos parasitas nas fezes, facilitando a contaminação ambiental. Outros geo-helmintos, como *Toxocara canis*, *Toxocara cati* e *A. caninum*, possuem animais domésticos como hospedeiros definitivos e a contaminação ambiental por fezes de cães e gatos facilita a ocorrência de zoonoses, como a larva *migrans*. As geo-helmintíases são endêmicas no Brasil, mas as informações sobre geo-helmintos no estado do Rio Grande do Sul são escassas e geralmente estão descritas na literatura de forma fragmentada. Dessa forma, este artigo revisa, integra e discute dados e informações sobre geo-helmintos no Rio Grande do Sul, com base em estudos realizados com amostras humanas, animais e ambientais, em uma estratégia alinhada à perspectiva de Saúde Única. Os potenciais impactos das alterações ambientais observadas no Rio Grande do Sul sobre a ocorrência das geo-helmintíases também são abordados neste artigo.

**Palavras-chave:** helmintos, meio ambiente, parasitas, saúde pública, zoonoses.

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**Abstract:** The intestinal helminths *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms *Ancylostoma duodenale* and *Necator americanus* have strong public health relevance in Brazil, being associated with nutritional deficiencies, gastrointestinal problems and deficits in child development. These species are collectively known as soil-transmitted helminths, as the soil plays an important role in the transmission and development of these parasites. Environmental contamination by soil-transmitted helminth eggs and larvae is particularly common in areas with problems in potable water distribution and/or in sewage treatment systems. Humans infected with *A. lumbricoides*, *T. trichiura* and hookworms can release a large amount of parasite eggs in their feces, facilitating environmental contamination. Other soil-transmitted helminths, such as *Toxocara canis*, *Toxocara cati* and *A. caninum*, have domestic animals as definitive hosts. Therefore, environmental contamination by feces of dogs and cats facilitates the occurrence of zoonoses, such as larva migrans. Soil-transmitted helminth infections are endemic in Brazil, but information on soil-transmitted helminths in the Rio Grande do Sul State is scarce and is usually described in the literature in a fragmented way. Thus, this article reviews, integrates and discusses data and information on soil-transmitted helminths in the Rio Grande do Sul, based on studies carried out with human, animal and environmental samples, in a strategy aligned with the One Health perspective. The potential impacts of environmental changes observed in the Rio Grande do Sul on the occurrence of soil-transmitted helminths are also addressed in this article.

**Keywords:** helminths, environment, parasites, public health, zoonoses.

## Introdução

### Aspectos básicos sobre os geo-helmintos

As geo-helmintíases representam um grupo de doenças parasitárias causadas pelos parasitas intestinais *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos, sendo *Ancylostoma duodenale* e *Necator americanus* as espécies de ancilostomídeos que apresentam maior relevância em termos de saúde pública no Brasil. Os geo-helmintos são nematódeos que apresentam parte do ciclo de desenvolvimento no ambiente, mais especificamente no solo (*geo*), de onde é derivado o termo “geo-helminto” (no inglês, *soil-transmitted helminth*). O solo contaminado com ovos e larvas desses parasitas possui papel importante na transmissão das geo-helmintíases para os humanos. Os humanos são os principais hospedeiros definitivos dos geo-helmintos, bem como seus reservatórios, não sendo incomum um mesmo indivíduo estar infectado por mais de uma espécie de geo-helminto, condição conhecida como poliparasitismo<sup>1,2</sup>.

Apesar de cada espécie de parasita apresentar particularidades biológicas e ecológicas, eles compartilham uma série de características: (I) apresentam semelhança na distribuição geográfica; (II) os grupos populacionais afetados pelos diferentes parasitas são semelhantes; (III) os tratamentos utilizados para as infecções são os mesmos; (IV) as técnicas utilizadas para o diagnóstico parasitológico são as mesmas ou similares; (V) as condições sócio-ambientais associadas à infecção são semelhantes; (VI) alguns efeitos da infecção sobre a saúde humana, como a anemia e problemas gastrointestinais, são comuns a diferentes geo-helmintos; e (VII), como mencionado anteriormente,

são helmintos que apresentam parte do ciclo de desenvolvimento no solo, o qual possui papel relevante na transmissão desses parasitas<sup>2</sup>.

Em decorrência da perda de massa magra, deficiências nutricionais e problemas gastrointestinais associados com a presença dos parasitas no trato gastrointestinal, as geohelmintíases podem causar anemia gestacional e perda de capacidade produtiva do indivíduo afetado na idade adulta. Porém, as geohelmintíases são mais preocupantes quando ocorrem em crianças, pois nesse grupo podem causar sérios problemas de desenvolvimento, afetando crescimento, aprendizado e cognição. A intensidade dos sinais e sintomas dessas parasitoses varia conforme fatores associados ao hospedeiro, como estado nutricional e características genéticas, e carga de infecção, geralmente significando o número de parasitas presentes no hospedeiro e indicada pelo número de ovos presentes nas fezes<sup>3-6</sup>. A **Figura 1** resume as principais consequências decorrentes da infecção por geo-helmintos.

### ***Ascaris lumbricoides***

*Ascaris lumbricoides* (no inglês, *roundworm*) é um parasita intestinal cosmopolita, apresenta aspecto alongado e cilíndrico e é popularmente conhecido no Brasil como “lombriga” (**Figura 2**). Nas formas adultas, os parasitas machos medem de 15 a 25 cm e as fêmeas de 20 a 40 cm<sup>7</sup>. A infecção por *A. lumbricoides* ocorre quando humanos ingerem acidentalmente os ovos do parasita, geralmente através do consumo de alimentos contaminados ou pelo contato com o solo onde os ovos estão depositados. A contaminação ambiental acontece através das fezes de um hospedeiro humano infectado. Um indivíduo parasitado por *A. lumbricoides* pode liberar no ambiente mais de 200 mil ovos por dia, valor este considerando-se um único parasita. Os ovos podem estar ou não fertilizados, dependendo da presença de parasitas de ambos os sexos no lúmen do hospedeiro. O desenvolvimento dos ovos fertilizado em ovos embrionados (contendo larvas infectantes) vai depender de condições ambientais apropriadas (umidade e temperatura do solo, entre outras)<sup>1,7,8</sup>.

Após serem ingeridos acidentalmente por um novo hospedeiro humano, os ovos embrionados (medindo 90µm x 45µm) de *A. lumbricoides* chegam até o trato gastrointestinal, onde as larvas emergem (medindo 250µm x 14µm) e penetram a parede intestinal, atingindo a circulação sanguínea e chegando até os pulmões. Alternativamente, as larvas podem atingir os pulmões através da migração visceral. Já nos pulmões, as larvas passam por um período obrigatório de maturação que dura de 10 a 14 dias. No tecido pulmonar, as larvas crescem consideravelmente e passam por uma mudança na sua “assinatura transcriptômica” (perfil de expressão de proteínas). Apesar da migração da larva para o tecido pulmonar ser essencial ao desenvolvimento do parasita, o motivo da maturação acontecer especificamente nos pulmões não está elucidado. Após esse período de maturação, as larvas penetram as paredes alveolares e migram até a faringe, sendo

deglutidas pelo hospedeiro. Após chegarem ao intestino delgado, as larvas podem se desenvolver em parasitas adultos (medindo em torno de 15cm), vivendo de um a três anos. Em caso de infecção severa, as larvas podem causar deficiências nutricionais, obstrução intestinal, alteração da microbiota intestinal, entre outros problemas. A infecção por parasitas de ambos os sexos permite o acasalamento e a liberação de ovos fertilizados nas fezes humanas, reiniciando o ciclo biológico do parasita<sup>1,3,7-10</sup>.



**Figura 1.** Principais consequências decorrentes da infecção por geo-helmintos. As formas adultas de *Ascaris lumbricoides*, *Trichuris trichiura* e um exemplar de ancilostomídeo (*Ancylostoma duodenale*) estão representados na figura. Figura elaborada pelos autores com o auxílio da plataforma Servier Medical Art (<https://smart.servier.com/>).



**Figura 2.** As formas adultas de *Ascaris lumbricoides*, *Trichuris trichiura* e um exemplar de ancilostomídeo (*Ancylostoma duodenale*) estão representados na figura, junto aos seus respectivos ovos. Aspectos humanos, ambientais e animais influenciam a distribuição e carga das estruturas parasitárias de *A. lumbricoides* (ovos), *T. trichiura* (ovos) e dos ancilostomídeos (ovos e larvas) nos biomas terrestres do Rio Grande do Sul (RS), assim como de geo-helminths zoonóticos como *Toxocara canis*, *Toxocara cati*, entre outros (não representados na figura). Fatores humanos, animais e ambientais também influenciam o risco de transmissão dos geo-helminths entre as populações humanas. Ressalta-se que características geoclimáticas, políticas e socioeconômicas também ajudam a explicar diferenças na prevalência das geo-helminthíases observadas em diferentes regiões do RS e em relação a outros estados brasileiros. Figura elaborada pelos autores com o auxílio das plataformas Servier Medical Art (<https://smart.servier.com/>) MapChart (<https://www.mapchart.net>).



### *Trichuris trichiura*

Assim como acontece com *A. lumbricoides*, a infecção por *T. trichiura* (no inglês, *whipworm*, verme “chicote” - em decorrência de seu aspecto morfológico; **Figura 2**) ocorre após ingestão acidental dos ovos dos parasitas presentes em alimentos ou pelo contato oral com o solo. A contaminação tanto do solo como de alimentos com os ovos de *T. trichiura* se dá pelas fezes de um hospedeiro humano parasitado<sup>1,11</sup>.

Os ovos de *T. trichiura* passam por um estágio de desenvolvimento no solo após serem liberados no ambiente, tornando-se ovos embrionados, contendo larvas infectantes. Após esses ovos serem ingeridos por um novo hospedeiro, as larvas eclodem no intestino delgado e maturam junto às vilosidades intestinais. Os parasitas adultos vivem no cécum e cólon ascendente, alimentando-se de sangue e nutrientes do hospedeiro. Um parasita adulto mede entre 2,5 e 4 cm e vive em torno de um ano. Parasitas de ambos os sexos são geralmente encontrados no intestino humano, permitindo o acasalamento. As fêmeas podem liberar entre 3 e 20 mil ovos por dia. Infecções severas podem causar anemia e problemas gastrointestinais, incluindo diarreia, dor abdominal e prolapso retal<sup>1,11,12</sup>.

### *Ancilostomídeos*

A infecção por ancilostomídeos (*Ancylostoma duodenale* e *Necator americanus*), conhecidos no inglês como *hookworms* (vermes “gancho” devido ao aspecto morfológico; **Figura 2**), acontece através da penetração de larvas dos parasitas na pele do hospedeiro humano. As larvas de ancilostomídeos são encontradas no solo e originam-se de ovos liberados no ambiente nas fezes de um hospedeiro humano parasitado. Após um período de maturação no solo sob condições de temperatura, sombreamento e umidade adequadas, larvas rhabditóides (de vida livre e não infectantes) eclodem dos ovos. As larvas rhabditóides podem se desenvolver em larvas filarióides (infectantes), processo também dependente de condições adequadas do solo. Novos hospedeiros humanos podem entrar em contato com as larvas filarióides ao andarem descalços no ambiente, sentarem no chão com as nádegas descobertas ou através da manipulação de solo ou vegetação contendo as larvas<sup>1,13,14</sup>.

Após penetrarem o tecido cutâneo, as larvas de ancilostomídeos atingem a circulação sanguínea, chegam até o coração e então acessam os pulmões, de onde migram até a faringe e são deglutidas pelo hospedeiro. As larvas desenvolvem-se em parasitas adultos após atingirem o intestino delgado, onde se fixam à parede intestinal, alimentando-se de sangue e tecidos do hospedeiro. Usualmente os parasitas adultos medem 2,5 cm e vivem de um a dois anos, mas períodos de vida maiores que esses podem ser observados. Na presença de parasitas de ambos os sexos, acontecerá o acasalamento, sendo que as fêmeas de ancilostomídeos podem liberar de

centenas a milhares de ovos a cada dia. A infecção intestinal por ancilostomídeos pode causar deficiências nutricionais (especialmente anemia por deficiência de ferro), complicações gastrointestinais e disfunções imunológicas<sup>1,13,14</sup>. Por fim, destaca-se que larvas de *A. duodenale* também podem ser acidentalmente ingeridas, causando náusea, vômitos, tosse, entre outros sinais e sintomas, condição conhecida como síndrome Wakana<sup>1</sup>.

### Outros geo-helminhos

Apesar de não fazerem parte do grupo clássico de geo-helminhos citados anteriormente, *Ancylostoma braziliense* e *A. caninum* são ancilostomídeos cujos ovos e larvas também podem ser encontrados no solo, pois possuem cães e gatos como hospedeiros definitivos, os quais contribuem para a contaminação ambiental por esses parasitas através da liberação de ovos nas fezes. Quando larvas de *A. braziliense* e *A. caninum* infetam humanos, esses ancilostomídeos não conseguem atingir o sistema gastrointestinal, afetando apenas o tecido cutâneo. As larvas migram pela epiderme, causando a doença conhecida como larva *migrans* cutânea, popularmente chamada no Brasil de “bicho geográfico”. A migração da larva sob a pele pode durar de semanas a meses, causando uma importante reação inflamatória e prurido intenso, provocando dor e atrapalhando o sono e atividades diurnas dos indivíduos afetados. Infecções bacterianas secundárias podem ocorrer na pele afetada por larva *migrans*<sup>15-17</sup>.

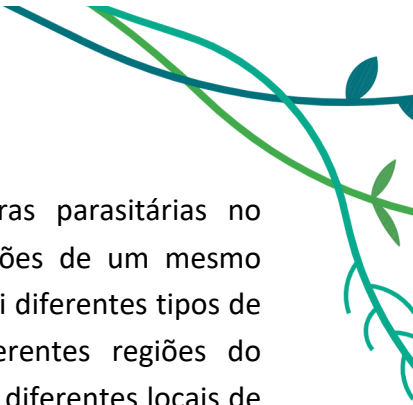
O nematódeo intestinal *Strongyloides stercoralis* também tem relevância epidemiológica no Brasil. Este parasita possui um ciclo de vida complexo e que pode ser consultado detalhadamente no site do *Center for Disease Control and Prevention*<sup>18</sup>. A infecção de humanos por *S. stercoralis* pode causar inflamação da mucosa intestinal entre outras complicações gastrointestinais, uma condição conhecida como estrongiloidíase<sup>1,18</sup>.

No Brasil, também possui importância epidemiológica a infecção de humanos por *Toxocara canis* e *Toxocara cati*, agentes causadores das doenças larva *migrans* visceral e larva *migrans* ocular<sup>3,19</sup>. Estruturas parasitárias de *S. stercoralis*, de parasitas do gênero *Toxocara*, entre outros, podem ser encontradas no solo e por isso também estes organismos são classificados como geo-helminhos<sup>3</sup>.

### Aspectos epidemiológicos e socioambientais

As geo-helminthiases ocorrem em diferentes países da América Latina, sendo endêmicas no Brasil<sup>4,20</sup>, com intensidade dos focos de infecção variando bastante entre diferentes regiões nacionais, especialmente em termos de prevalência em crianças<sup>20</sup>. Essas diferenças podem ser evidentes mesmo em locais próximos. Considerando que o tipo de solo e suas características (temperatura,





umidade) influenciam a distribuição, sobrevivência e carga das estruturas parasitárias no ambientes<sup>21-23</sup>, o risco de infecções por geo-helminhos em diferentes regiões de um mesmo município naturalmente não são as mesmas. Por exemplo, Porto Alegre possui diferentes tipos de solos (argissolos, planossolos, neossolos flúvicos, entre outros) em diferentes regiões do município<sup>24,25</sup>, o que pode explicar diferenças nos níveis de contaminação em diferentes locais de Porto Alegre.

As geo-helminthiases são doenças negligenciadas associadas com deficiências na infraestrutura de distribuição de água potável e com problemas de saneamento ambiental e moradia, sendo consideradas no Brasil como “doenças dos pobres”<sup>2,26</sup>, apesar de não serem restritas a esta parcela da população. A prevalência de infecção por geo-helminhos no Brasil apresenta importante redução desde a década de 1990, especialmente considerando *A. lumbricoides* e ancilostomídeos<sup>27</sup>. Ações de controle integrando melhoria das condições de saneamento ambiental, educação sanitária e o uso de anti-helmínticos (albendazol, mebendazol), especialmente em crianças em idade escolar, contribuíram para essa redução<sup>2,27,28</sup>.

A prevalência das geo-helminthiases está associada com o nível de desenvolvimento socioeconômico de cada país<sup>29</sup>. Acesso a água potável e saneamento foram recentemente observados como importantes fatores protetores contra as geo-helminthiases no Brasil, com base em dados do período de 2010 a 2015<sup>30</sup>. Porém, as geo-helminthiases continuam representando importantes problemas de saúde pública no Brasil<sup>31</sup>, trazendo impactos diretos sobre o bem estar da população e prejuízos econômicos e sociais em decorrência da perda de produtividade e desenvolvimento infantil deficitário.

Uma meta-análise publicada em 2013 por Chammartin et al.<sup>32</sup> estimou prevalências de infecção de 14,3% para *A. lumbricoides*, 12,3% para ancilostomídeos e 10,1% para *T. trichiura* entre a população Brasileira, considerando o período de 2005 em diante e com dados de prevalência ajustados pela população<sup>32</sup>. Essa meta-análise possuiu algumas limitações, especificamente na seleção dos estudos<sup>33</sup> e uso de dados obtidos através de diferentes métodos diagnósticos<sup>34</sup>, o que pode ter influenciado as taxas de prevalência. Já em um estudo publicado em 2014 e utilizando dados a partir de 2010 extraídos da “*Global Neglected Tropical Diseases Database*”, Chammartin et al.<sup>27</sup> estimaram prevalências (ou % de população sob risco) de 3,6% para *A. lumbricoides*, 1,7% para ancilostomídeos e 1,4% para *T. trichiura* entre a população Brasileira. De acordo com estas estimativas, aproximadamente 6% da população nacional estaria sob risco em relação a geo-helminthiases<sup>27</sup>.

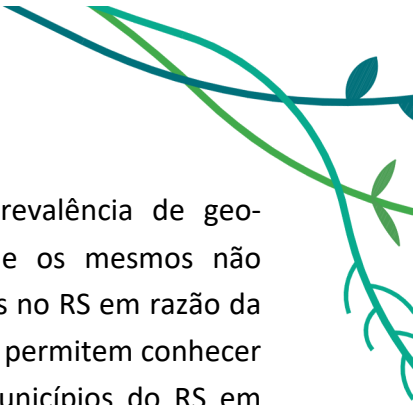
A distribuição dos casos de geo-helminthiases não é homogênea no Brasil, sendo a prevalência particularmente alta em algumas regiões do país. Por exemplo, as taxas de prevalência na região Amazônica foram recentemente estimadas em 42,6% para *T. trichiura*, 24,4% para *A. lumbricoides*

e 9,0% para ancilostomídeos<sup>26</sup>. Em termos de mortalidade, mulheres, crianças menores de 10 anos, Indígenas e residentes do Nordeste são os grupos mais impactados pelos efeitos das geo-helminthiases no Brasil<sup>35</sup>.

O “Inquérito Nacional de Prevalência da Esquistossomose mansoni e Geo-helminthoses”, publicado em 2018, avaliou a ocorrência da esquistossomose, tricuriase, ancilostomíase e ascaridíase em todas as 27 Unidades da Federação Brasileiras, apresentando dados referentes ao período entre 2010 e 2015<sup>31</sup>. As parasitoses foram avaliadas em amostras de fezes através do método de diagnóstico parasitológico Kato-Katz<sup>36</sup>. Foram utilizadas amostras de mais de 197 mil crianças e adolescentes entre 7 e 17 anos, de ambos os sexos, residentes em 521 municípios Brasileiros. No entanto, a adesão do estado do Rio Grande do Sul (RS) ao Inquérito foi baixa, atingindo apenas 36,9% de amostras avaliadas em relação ao total previsto. Apenas 14 cidades do RS foram amostradas, com um total de 1.611 amostras avaliadas<sup>31</sup>. Em nível nacional, 2,73% das amostras foram positivas para ovos de ancilostomídeos, 6,00% das amostras apresentavam ovos de *A. lumbricoides* e 5,41% das amostras foram positivas para *T. trichiura*. A **Tabela 1** apresenta uma compilação das proporções de amostras positivas para geo-helminthos nas cinco Regiões Brasileiras, conforme dados obtidos por Katz<sup>31</sup>. No RS, as taxas de amostras positivas foram de 0,01% para ancilostomídeos, 0,56% para *A. lumbricoides* e 0,68% para *T. trichiura*<sup>31</sup>.

**Tabela 1.** Proporções de amostras positivas para geo-helminthos nas cinco Regiões Brasileiras (dados obtidos do inquérito coprológico nacional publicado por Katz<sup>31</sup>).

Geo-helmintho	Região	Total de amostras analisadas	Amostras positivas	Proporção de amostras positivas	Intervalo de confiança (95%)
Ancilostomídeos	Norte	18210	547	4,90%	0,90-8,89
	Nordeste	111606	4203	4,53%	3,26-5,81
	Sudeste	44473	397	0,69%	0,36-1,02
	Sul	14146	13	0,08%	0,00-0,18
	Centro-Oeste	9129	32	0,25%	0,07-0,44
<i>Ascaris lumbricoides</i>	Norte	18210	1348	10,80%	6,62-14,99
	Nordeste	111606	8945	8,26%	6,68-9,83
	Sudeste	44473	893	2,04%	1,10-2,98
	Sul	14146	289	3,71%	1,87-5,54
	Centro-Oeste	9129	46	1,19%	0,09-2,29
<i>Trichuris trichiura</i>	Norte	18210	1637	15,08%	6,39-23,77
	Nordeste	111606	8074	5,93%	4,69-7,16
	Sudeste	44473	613	1,77%	0,80-2,94
	Sul	14146	314	4,10%	2,01-6,19
	Centro-Oeste	9129	16	0,35%	0,00-0,91



Apesar dos dados obtidos no Inquérito<sup>31</sup> indicarem uma baixa prevalência de geohelmintíases no RS em comparação aos dados nacionais, é possível que os mesmos não representem de forma adequada a situação epidemiológica dessas parasitoses no RS em razão da baixa adesão do estado ao estudo. Além disso, os dados em nível estadual não permitem conhecer de forma completa a situação epidemiológica das diferentes regiões ou municípios do RS em decorrência do pequeno número de cidades amostradas ( $n=14$ ) e também devido ao limitado número amostral avaliado em alguns municípios. Por exemplo, apenas 15 amostras de fezes foram submetidas ao exame parasitológico em Porto Alegre, capital do RS<sup>31</sup>.

As taxas de mortalidade das geohelmintíases em diferentes municípios do RS são heterogêneas<sup>35</sup>. Além disso, um saneamento ambiental deficitário é observado em diferentes municípios do RS<sup>37</sup>, mesmo em locais considerados como “bolsões de desenvolvimento”. Porto Alegre, por exemplo, apresenta importantes problemas nos sistemas de distribuição de água potável e coleta de lixo e esgoto, com esses serviços ocorrendo de forma bastante heterogênea nas diferentes regiões da cidade<sup>38</sup>.

A ocorrência de larva *migrans* cutânea está associada com a contaminação ambiental por fezes de cães e gatos em áreas de recreação infantil, como caixas de areia de praças, escolas e creches. No Brasil, a doença ocorre geralmente em crianças que fazem uso desses espaços e entram em contato com larvas de *A. braziliense* ou *A. caninum*<sup>39</sup>. Porém, a infecção pode ocorrer também em adultos expostos às larvas de *Ancylostoma* spp. presentes em solo contaminado de praias ou ambientes comunitários onde há circulação de animais domésticos<sup>16</sup>. As informações epidemiológicas sobre casos de larva *migrans* cutânea são ainda bastante deficitárias, tanto em nível nacional como em nível estadual. Em um estudo envolvendo 1.185 habitantes de uma comunidade do Ceará, a prevalência de larva *migrans* cutânea foi de 3,1%<sup>40</sup>. Não foram encontrados dados de prevalência da doença em humanos no RS.

Considerando a escassez de dados em nível estadual e os problemas de saneamento ambiental observados no RS, é possível que as geohelmintíases representem um problema maior do que o atualmente descrito para esse estado. Dessa forma, o objetivo deste artigo é revisar dados sobre geohelminthos no RS, sintetizando as informações sobre esses parasitas no contexto estadual. Destacamos que este artigo trará dados principalmente sobre os geohelminthos intestinais humanos de maior importância no Brasil (*A. lumbricoides*, *T. trichiura*, *A. duodenale* e *N. americanus*). Algumas informações pontuais sobre geohelminthos zoonóticos (como *A. braziliense*, *A. caninum* e *Toxocara* spp.) serão ocasionalmente apresentadas.

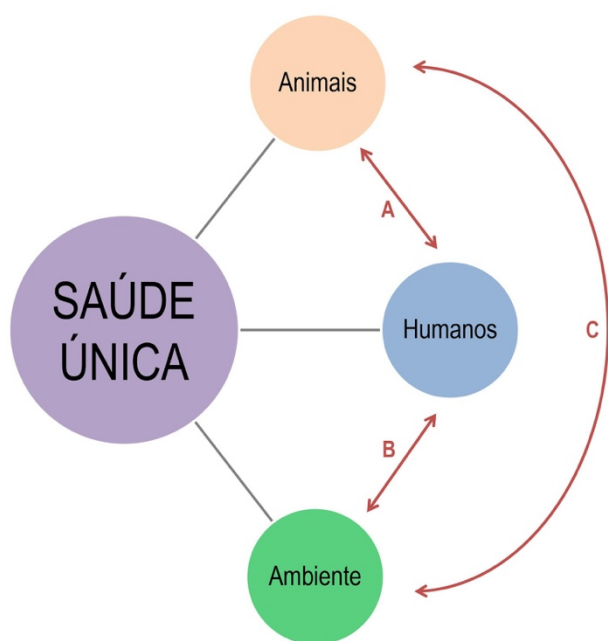
Considerando que as geohelmintíases envolvem fatores humanos, ambientais e animais (**Figura 3**), este artigo abordará estudos que investigaram a presença de estruturas parasitárias (ovos e larvas) em amostras de fezes humanas (considerando *A. lumbricoides*, *T. trichiura*, *A.*

*duodenale* e *N. americanus*) e animais (considerando geo-helminhos zoonóticos), assim como em amostras ambientais (solo, alimentos/vegetais e fezes encontradas no ambiente). A abordagem deste artigo está alinhada com a perspectiva de Saúde Única (*One Health*), pois discute as geohelmintíases no contexto das interfaces e relações entre humanos, animais e ambiente<sup>41-43</sup>.

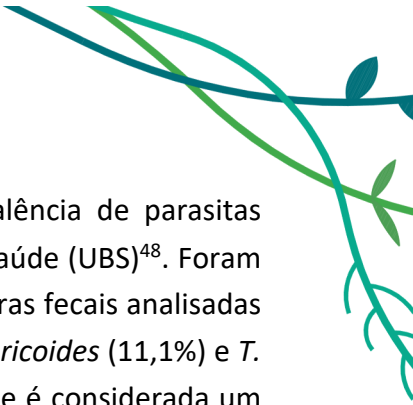
## Estudos no estado do Rio Grande do Sul

### Humanos

Alguns autores investigaram a prevalência de geo-helminthíases em municípios do RS, principalmente em crianças em idade escolar, pois este grupo é altamente susceptível aos geo-helminthos. Crianças também eliminam grande quantidade de ovos nas fezes, desempenhando um papel epidemiológico importante na disseminação dessas parasitoses<sup>2</sup>. Berne et al.<sup>44</sup> avaliaram a presença de parasitas em crianças atendidas em creches públicas do município de Rio Grande, as quais atendem famílias carentes de diferentes bairros da zona urbana. Das 165 crianças avaliadas, 106 estavam parasitadas por enteroparasitas. Em relação aos helmintos, a prevalência encontrada de *T. trichiura* foi de 24,2% e a de *A. lumbricoides* foi de 22,4%. Indivíduos do sexo masculino apresentaram maior frequência de parasitoses<sup>44</sup>. Ovos de *A. lumbricoides* e *T. trichiura* são frequentemente observados em associação em amostras fecais. Isso ocorre pela similaridade entre os ciclos de vida desses parasitas e pela deposição de ovos em grandes quantidades nas fezes, facilitando a contaminação ambiental de áreas como o peridomicílio<sup>45,46</sup>. Além disso, os fatores de risco para infecção por esses parasitas são bastante similares, compreendendo principalmente condições ambientais e comportamentais humanas<sup>47</sup>.



**Figura 3.** Dinâmica de transmissão de parasitas, incluindo geo-helminthos, entre humanos, animais e ambiente. A: parasitas podem ser transmitidos, direta ou indiretamente, de animais para humanos (antropozoonoses ou zoonoses) e de humanos para animais (zooantroponoses). B: humanos podem contaminar o ambiente com parasitas (por exemplo, com fezes contaminadas com ovos de geo-helminthos), sendo que o ambiente pode abrigar parasitas patogênicos aos humanos. C: animais podem contaminar o ambiente com parasitas, sendo que o ambiente pode abrigar parasitas patogênicos aos animais. Figura elaborada pelos autores.



Outro estudo, também conduzido em Rio Grande, analisou a prevalência de parasitas intestinais em crianças pré-escolares atendidas em seis Unidades Básicas de Saúde (UBS)<sup>48</sup>. Foram detectados ovos e cistos de enteroparasitas patogênicos em 17,4% das amostras fecais analisadas entre 2009 e 2010. Os ovos dos nematóides mais frequentes foram de *A. lumbricoides* (11,1%) e *T. trichiura* (6,3%). As UBS estão inseridas na Estratégia de Saúde de Família, que é considerada um dos principais serviços de promoção de saúde entre a população brasileira. Os dados do estudo<sup>48</sup> evidenciaram a necessidade de avaliação das condições sanitárias e de melhorias nas estratégias de controle das geo-helminthíases entre a população avaliada.

Em Uruguaiana, dois estudos avaliaram a presença de parasitoses intestinais em crianças. Chaves et al.<sup>49</sup> estudaram sete creches do município, seis delas em áreas periféricas, nos anos de 2002 e 2003. Observou-se uma porcentagem de 38,4% de crianças parasitadas, sendo *A. lumbricoides* presente em 22,0% e *T. trichiura* em 2,0% das amostras positivas para parasitas. Os dados podem ser interpretados como um reflexo da precariedade de moradia das crianças avaliadas, uma vez que muitas delas residiam em locais com saneamento precário e problemas socioeconômicos. Já Figueiredo e Querol<sup>50</sup> investigaram parasitoses em crianças entre 4 e 12 anos de idade e em funcionários que manipulavam alimentos em um centro socioeducativo. Além das análises parasitológicas, informações socioeconômicas dos participantes foram coletadas. Das 88 amostras analisadas, 37 (42%) delas foram positivas para infecção, sendo *A. lumbricoides* (21,6%) e *T. trichiura* (16,2%) os geo-helminthos mais frequentes. O gênero *Acylostoma* apresentou frequência de 5,4%. A maior parte das crianças afetadas fazia parte de famílias de baixa renda, eram filhas de mães com baixa escolaridade e viviam em moradias com saneamento precário<sup>50</sup>.

Nagel et al.<sup>51</sup> também encontraram uma associação entre indivíduos parasitados, baixa renda familiar e baixa escolaridade materna no município de Palmeira das Missões. O estudo verificou uma prevalência de parasitoses intestinais de 59,3% entre crianças em idade escolar, sendo que *A. lumbricoides* foi o parasita mais frequente (55,6%). *Trichuris trichiura* foi observado em 2,4% das amostras<sup>51</sup>. Poucos estudos analisam a prevalência de infecção por enteroparasitas na região norte do RS, onde Palmeira das Missões se localiza. Os municípios de Santo Ângelo e Campo Novo, também localizados nessa região, apresentaram prevalências de infecção por parasitas intestinais bastante altas: 63,6%<sup>52</sup> e 80%<sup>53</sup>, respectivamente. *Ascaris lumbricoides* esteve presente nas amostras de ambas as cidades<sup>52,53</sup>, porém a análise na cidade de Campo Novo diferenciou-se por revelar uma taxa elevada de infecção por *Ancylostoma* spp., com uma frequência de 42,1%<sup>53</sup>.

Reuter et al.<sup>54</sup> avaliaram a prevalência de enteroparasitas em crianças de 0 a 5 anos que frequentavam creches do município de Santa Cruz do Sul. Foi observada uma frequência de 32,2% de amostras positivas para parasitoses intestinais, sendo que *A. lumbricoides* estava presente em 10% delas. Em sua maioria, os pais de crianças parasitadas não possuíam ensino fundamental

completo. Embora grande parte das famílias residisse em casas com acesso à rede pública de esgoto, o tipo de esgoto classificado como “fossa” foi reportado em 35,5% das famílias<sup>54</sup>.

As cidades da serra gaúcha geralmente apresentam prevalências menores de infecção por parasitoses intestinais quando comparado a outras regiões do estado. O município de São Marcos apresentou uma prevalência de 5,79% em escolares<sup>55</sup>. Já as prevalências em Flores da Cunha<sup>56</sup> e em Ipê<sup>57</sup> foram de 10% e 8,1%, respectivamente. A menor prevalência em algumas cidades da serra gaúcha pode estar relacionada com a concentração de cidades com Índice de Desenvolvimento Humano (IDH) elevado, o que reflete os investimentos em infraestrutura e qualidade de vida, mas também pode ser explicado por questões climáticas, já que cidades serranas são caracterizadas por possuírem inverno mais rigoroso. Porém, mais estudos são necessários para corroborar ou não estas hipóteses.

Em Caxias do Sul, município que também faz parte da serra gaúcha, um estudo avaliou escolares na faixa de 6 a 14 anos, analisando amostras coprológicas durante 35 anos<sup>46</sup>. No período entre 1969 e 2004, observou-se uma prevalência de 58% para enteroparasitas, com helmintos presentes em 65,1% das amostras positivas. Os geo-helmintos mais frequentes foram *A. lumbricoides* (47%) e *T. trichiura* (36%). *Ancylostomidae* também foi observado, porém em uma frequência relativamente menor (3,4%). A prevalência de infecções diminuiu consideravelmente durante os anos. Especificamente em 1969 observou-se 89% de amostras positivas para enteroparasitas, enquanto que em 2004 a taxa das amostras diminuiu para 37%, apontando um decréscimo médio na prevalência de 1,4% ao ano. *Ascaris* teve uma redução significativa de 61% para 26% e *Trichuris* diminuiu de 38% para 18%<sup>46</sup>. Interessantemente, não foi observada diferença significativa de infecção comparando zonas rural e urbana. Isso provavelmente reflete o fato de a zona rural de Caxias do Sul ser composta por pequenas propriedades agrícolas que possuem boas condições sanitárias, enquanto a periferia da zona urbana enfrenta problemas referentes ao saneamento. É possível que o decréscimo das parasitoses observado ao longo dos anos tenha relação com a melhoria da infraestrutura de saneamento e habitação, bem como ações de educação em saúde, medidas que reverberaram sobre a saúde pública<sup>46</sup>.

Porto Alegre, capital do RS, apresenta grandes desafios em relação às geo-helmintíases e saneamento. A cidade ocupa a 43ª posição no Ranking do Saneamento do Instituto Trata Brasil em 2022<sup>58</sup>. Embora esteja em primeiro lugar entre as cidades do estado, está distante das melhores posições no Brasil, principalmente porque parte da população do município ainda não é atendida pela coleta e tratamento de esgoto<sup>58</sup>. Um estudo realizado na periferia de Porto Alegre analisou a frequência de parasitoses intestinais em escolares, sendo que 36% das amostras apresentaram resultados positivos para um ou mais parasita, com *A. lumbricoides* presente em 50,7% e *T. trichiura* em 24,6% das amostras positivas<sup>59</sup>. Costa et al.<sup>60</sup> avaliaram a prevalência de parasitoses em crianças de 12 a 16 meses atendidas em unidades de saúde de Porto Alegre. A maioria das famílias



participantes do estudo vivia em condições sociais precárias. Apesar disso, a prevalência encontrada nessa faixa etária foi de 6,8%, sugerindo que esse grupo não é de risco para infecções parasitárias, possivelmente pelo limitado contato direto com o ambiente<sup>60</sup>. Porém, é fundamental interpretar esse resultado dentro do contexto urbano avaliado no estudo.

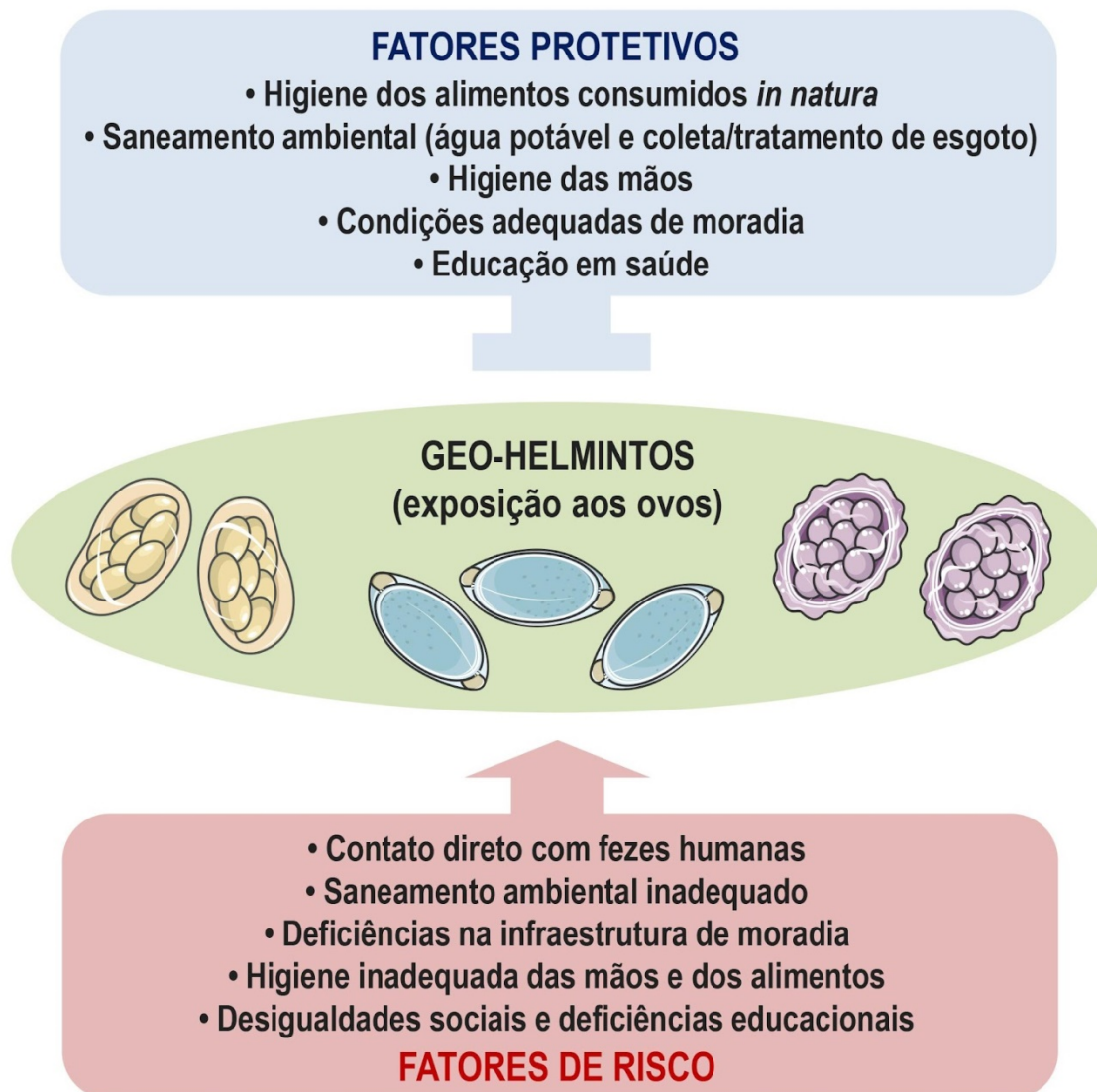
A população indígena também é uma população de risco para as geo-helmintíases, sendo ainda negligenciada em termos de medidas de saúde pública. Mundialmente, populações indígenas possuem uma elevada carga de doenças associadas com a pobreza, condições sanitárias precárias e contaminação ambiental<sup>61</sup>. A população Guarani, que se distribui entre as regiões Sul e Sudeste do Brasil, possui altas taxas de nascimento e mortalidade, com baixa expectativa de vida ao nascer<sup>62,63</sup>. Em Porto Alegre, um estudo avaliou a prevalência de parasitas intestinais em uma comunidade Mbyá-Guarani<sup>63</sup>. As parasitoses foram observadas em 88,7% da população analisada, sendo 45,5% referentes a poliparasitose, taxas estas bastante elevadas. Entre crianças na faixa de 1 a 12 anos, a prevalência chegou a 90,5%. Nesse grupo, *A. lumbricoides* apresentou frequência de 38%, enquanto que as frequências de *T. trichiura* e ancilostomídeos foram de 11,9% e 7,1%, respectivamente. Diversos fatores podem estar relacionados com a ocorrência de parasitismo em comunidades indígenas, como andar descalço, a defecação em locais próximos às áreas de convivência e hábitos de higiene que não incluem lavar as mãos frequentemente nem a proteção de utensílios utilizados nas refeições, as quais são geralmente preparadas junto ao chão<sup>63</sup>.

A variabilidade dos achados obtidos pelos estudos mencionados anteriormente pode estar relacionada com diferenças biológicas, socioeconômicas e culturais das diferentes populações analisadas. Os principais fatores de risco e protetivos em relação à infecção por geo-helminintos estão compilados na **Figura 4**. As estratégias de controle das geo-helmintíases nas populações humanas devem considerar esses fatores de forma integrada.

## Animais


Os animais domésticos, principalmente cães e gatos, possuem contato direto com o ser humano, frequentando espaços como casas, parques, praças e praias. Esses animais, principalmente aqueles considerados “de rua”, são frequentemente infectados por parasitas zoonóticos, representando um problema de saúde pública<sup>64</sup>. Animais domésticos circulam entre ambientes residenciais, áreas públicas e bordas de mata, eliminando ovos de parasitas nesses diferentes ambientes através das fezes, facilitando a ocorrência de doenças zoonóticas<sup>65</sup>. As doenças helmínticas transmitidas por cães e gatos incluem larva *migrans* visceral, causadas por *Toxocara*, e larva *migrans* cutânea, causada por *A. braziliense* e *A. canium*. A transmissão pode ocorrer por contato direto com o animal ou por contato indireto, através, por exemplo, de fezes ou alimento e água contaminados. A prevenção dessas zoonoses inclui testagem e tratamento dos animais e cuidados relacionados à higiene,

principalmente em locais de acesso público, com o objetivo de diminuir a contaminação ambiental<sup>66</sup>.



**Figura 4.** Principais fatores de risco e protetivos relacionados à infecção por geo-helmintos. Ovos de *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos estão representados na figura. Figura elaborada pelos autores com o auxílio da plataforma Servier Medical Art (<https://smart.servier.com/>).





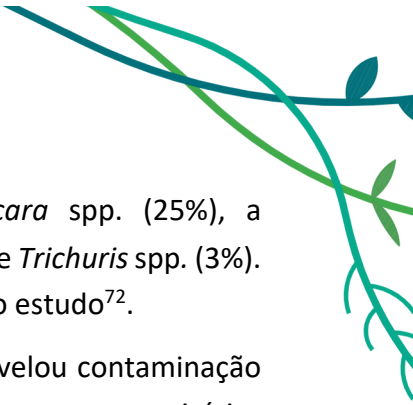
Poucos estudos avaliaram o potencial zoonótico de amostras fecais de animais domésticos em cidades do RS. Em Porto Alegre<sup>67</sup>, a avaliação da presença de parasitas intestinais em cães e gatos sob cuidados veterinários revelou resultados positivos para a presença de pelo menos um parasita em 26,6% dos cães e 20,5% dos gatos amostrados, sendo que os gêneros *Ancylostoma* e *Toxocara* estavam entre os mais frequentes nas amostras analisadas<sup>67</sup>.

Em Santa Maria, Pivoto et al.<sup>68</sup> analisaram a ocorrência de parasitas gastrointestinais em gatos urbanos domiciliados e fatores de riscos associados com a infecção. Além do exame coproparasitológico, foram aplicados questionários epidemiológicos sobre hábitos e manejo dos animais atendidos. Do total de amostras analisadas, 47,1% delas foram positivas para pelo menos um parasita intestinal. Os gêneros *Toxocara* e *Ancylostoma* foram observados em 18,8% e 2,6% dos animais monoinfectados, respectivamente. O estudo relatou uma associação entre parasitoses nos gatos com baixa escolaridade dos proprietários e frequência de tratamentos antiparasitários<sup>68</sup>.

### **Ambiente: solo, fezes e alimentos**

O ambiente faz parte da rota de transmissão de diversas doenças parasitárias, especialmente em contextos de saneamento precário<sup>2</sup>. O entendimento de como e onde as infecções por geohelmintos mais ocorrem (domicílio, escolas, parques, praias, entre outros) é bastante incompleto e tema de discussão. Conhecer os níveis de contaminação ambiental em diferentes locais e contextos ecológicos contribui para o melhor entendimento dos riscos aos quais as populações estão expostas, possibilitando a criação de estratégias de prevenção<sup>69</sup>. Locais como praças e parques geralmente apresentam grande contaminação por geohelmintos em decorrência da presença de animais domésticos<sup>64</sup>. Além disso, no Brasil é comum que pessoas em situação de rua utilizem locais públicos como moradia, principalmente nas áreas centrais das cidades, contribuindo para a contaminação ambiental em decorrência de fezes humanas depositadas no ambiente<sup>70</sup>. A contaminação do solo e da água por excretas humanas e animais facilita a ocorrência de doenças parasitárias, principalmente em indivíduos que possuem contato direto e duradouro com o ambiente. Este fator é ainda mais preocupante em escolas e creches, onde crianças brincam em áreas de recreação em contato frequente e intenso com o solo, sendo inclusive comum a geofagia (ingestão de terra, argila ou areia)<sup>71</sup>.

Estudos sobre contaminação ambiental por geohelmintos contribuem para avaliar o risco de infecção ao qual a população está sujeita. No RS, embora não existam dados oficiais a respeito da contaminação ambiental por geohelmintos, alguns estudos sugerem as taxas de contaminação em diferentes municípios. Prestes et al.<sup>72</sup> analisaram a presença de geohelmintos em amostras de solo de dez praças distribuídas entre os municípios de Capão do Leão, Cerrito, Jaguarão, Turuçu, Pedro Osório e São Lourenço do Sul, todos localizados na região sul do RS. As dez praças analisadas

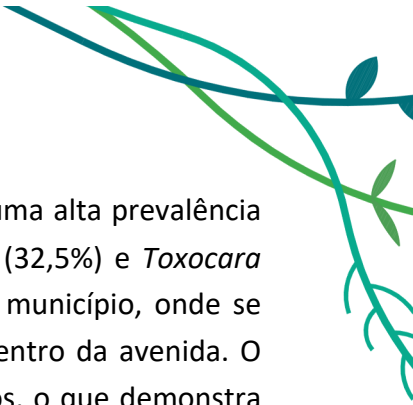


apresentaram contaminação, sendo os parasitas mais frequentes: *Toxocara* spp. (25%), a superfamília Strongyloidea - possíveis ancilostomídeos (11%), *Ascaris* spp. (4%) e *Trichuris* spp. (3%). Amostras contaminadas por mais de uma espécie de parasita foram comuns no estudo<sup>72</sup>.

Uma análise dos solos de dezoito espaços públicos de Porto Alegre revelou contaminação ambiental em 100% dos locais avaliados<sup>73</sup>. Ovos do gênero *Ascaris* foram as estruturas parasitárias observadas com maior frequência (10,2%), seguidas por ovos dos gêneros *Trichuris* (4,4%) e *Toxocara* (4,2%). Os locais que apresentaram maior frequência de *Ascaris* foram parques com menor incidência de luz solar. As estruturas parasitárias foram mais abundantes em meses de temperaturas amenas, provavelmente em decorrência da menor dessecação das mesmas pelo calor. Os ovos de *Trichuris* são mais resistentes às intempéries, o que provavelmente está relacionado com sua casca mais espessa, a qual dificulta a dessecação e ajuda a explicar a prevalência encontrada no estudo<sup>73</sup>.

A “praia” de Ipanema é um local público frequentemente utilizado como balneário durante o verão em Porto Alegre, principalmente pela população de maior vulnerabilidade socioeconômica<sup>74</sup>. Matesco et al.<sup>74</sup> analisaram amostras de areia coletadas nesse local e encontraram contaminação por geo-helminhos em 13,3% delas. Além disso, 33,9% de amostras de fezes coletadas no local apresentaram contaminação por geo-helminhos. É comum que fezes apresentem maior prevalência de contaminação por serem materiais mais concentrados e de origem animal, enquanto que em amostras de solo as estruturas parasitárias estão dispersas e expostas a condições que facilitam sua dessecação e destruição<sup>74</sup>.

No município de Pelotas, a análise de diferentes locais públicos revelou contaminação por geo-helminhos em 44% de amostras de solo, com múltiplos parasitas presentes em 16,5% delas<sup>75</sup>. Todos os parques amostrados exibiam alta circulação de pessoas e animais e apresentaram algum helminto com potencial zoonótico. Os ancilostomídeos foram os geo-helminhos mais frequentes (13,5%). Ovos dos gêneros *Toxocara*, *Trichuris* e *Ascaris* também foram observados, mas em menores frequências. A alta circulação de animais nos parques analisados ajuda a explicar a ocorrência de parasitas dos gêneros *Toxocara* e *Ancylostoma* observados nas amostras<sup>75</sup>. Também em Pelotas, a presença de estruturas parasitárias foi avaliada em amostras de solo da orla das praias do Laranjal, na Laguna dos Patos<sup>76</sup>. Das seis praças analisadas, quatro apresentaram ovos de helmintos, sendo o grupo dos ancilostomídeos um dos mais frequente nas amostras. Locais altamente turísticos como a Laguna dos Patos são frequentemente limpos e novas camadas de areia são recolocadas, o que pode sugerir menores taxas de contaminação ambiental por geo-helminhos. Ainda assim, os autores observaram animais “de rua” em todos os locais analisados, sendo que 66,7% desses locais apresentaram contaminação do solo por helmintos com potencial zoonótico<sup>76</sup>.

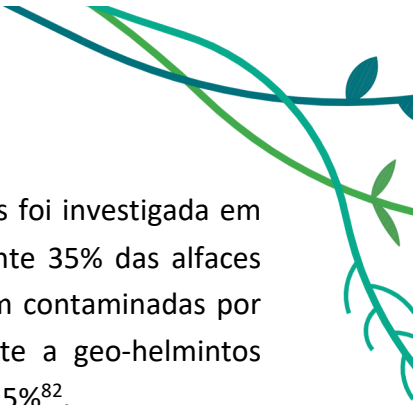


No Balneário Cassino (município de Rio Grande), Scaini et al.<sup>77</sup> encontraram uma alta prevalência de contaminação ambiental por *Ancylostoma* (71,3%), seguido por *Trichuris* (32,5%) e *Toxocara* (9,3%), com base na análise de fezes de cães presentes na área central do município, onde se encontram duas praças de recreação infantil e uma via para pedestres no centro da avenida. O estudo também encontrou a presença de larvas filarióides de ancilostomídeos, o que demonstra condições favoráveis para a infecção humana<sup>77</sup>.

Em uma análise de fezes caninas coletadas em locais próximos a escolas de cinco bairros de Pelotas, Mello et al.<sup>78</sup> observaram contaminação em todos os bairros, sendo que 74,7% das amostras foram positivas para um ou mais gêneros de helmintos. O mais observado foi *Ancylostoma* (93,2%), seguido por *Trichuris* (18,6%) e *Toxocara* (11,9%). A maior prevalência encontrada (80%) foi em uma área periférica da cidade, enquanto que a menor prevalência de contaminação ocorreu na região central (60%)<sup>78</sup>.

Padilha et al.<sup>79</sup> analisaram a ocorrência de parasitas com potencial zoonótico em caixas de areia utilizadas para recreação infantil em dois municípios do RS. De 100 amostras analisadas, 55% foram positivas para estruturas parasitárias. As amostras de ambos municípios apresentaram ovos e larvas de ancilostomídeos, demonstrando risco de infecção infantil<sup>79</sup>. Em Uruguaiana, Figueiredo et al.<sup>80</sup> avaliaram a presença de geo-helmintos em caixas de areia em sete escolas de educação infantil. Todas amostras apresentaram contaminação por ovos e larvas de parasitas, sendo os ancilostomídeos os mais frequentes (19,2%). Os gêneros *Toxocara*, *Ascaris* e *Trichuris* apresentaram frequências de 7,7%, 3,1% e 0,8%, respectivamente. Todas as escolas analisadas eram cercadas por muros ou telas, porém falhas no cercamento (permitindo a circulação de animais) e insuficiência na frequência de substituição da areia de recreação podem explicar a ocorrência de geo-helmintos nas amostras<sup>80</sup>.

O consumo de frutas e verduras cruas é incentivado por autoridades de saúde em decorrência dos benefícios nutricionais. Porém, o consumo de vegetais crus pode favorecer a transmissão de patógenos associados a doenças entéricas, incluindo os geo-helmintos. A agricultura familiar é uma prática bastante comum na região noroeste do RS. Tradicionalmente, pés de alface são plantados diretamente em contato com o solo, o que possibilita a contaminação por geo-helmintos através de irrigação por água ou pelo uso de fertilizantes orgânicos contaminados, por fezes de animais circulantes ou pela falta de condições higiênicas durante a manipulação desses alimentos<sup>81</sup>. Um estudo<sup>81</sup> realizado em oito municípios da região noroeste do RS avaliou a contaminação de alfaces vendidas em mercados e feiras locais. Entre 80 amostras analisadas, 22,9% delas apresentaram contaminação com potencial zoonótico, sendo Ancylostomatidae o grupo taxonômico mais frequente (8,75%). Ovos dos gêneros *Toxocara*, *Trichuris* e *Ascaris* também foram encontrados nas amostras<sup>81</sup>.




Em Itaqui, município da fronteira oeste do RS, a presença de parasitas foi investigada em amostras de alface de 30 mercados e 30 produtores locais. Aproximadamente 35% das alfaces encontradas nos mercados e 63% das amostras de produtores locais estavam contaminadas por uma ou mais espécies de parasita. O gênero *Ascaris* foi o único referente a geo-helmintos observado, estando presente em alfaces de ambas as fontes em frequência de 5%<sup>82</sup>.

Um estudo realizado em Pelotas investigou diferentes parasitas em amostras de alface, rúcula e agrião provenientes de supermercados e feiras livres<sup>83</sup>. Das 100 amostras analisadas, 29% estavam contaminadas por algum tipo de parasita, especialmente a rúcula (42,3% de amostras contaminadas). Os geo-helmintos observados foram larvas de ancilostomídeos e ovos dos gêneros *Toxocara* e *Ascaris*<sup>83</sup>. O estudo de estruturas parasitárias presentes em alimentos é relevante porque indica os riscos associados à contaminação por via oral através de alimentos de fácil acesso pela população. Esses estudos também sugerem as condições e práticas do cultivo que possibilitam a transmissão de parasitoses intestinais, incluindo as geo-helmintíases. A realização de novos estudos no RS investigando a presença de estruturas parasitárias em vegetais vendidos em mercados e feiras livres também pode contribuir para a melhoria das práticas de cultivo desses alimentos no estado.

## Desequilíbrios ambientais e geo-helmintíases no Rio Grande do Sul

Além do bioma Marinho Costeiro, dois biomas terrestres dominam a paisagem do RS, o Pampa e a Mata Atlântica<sup>84</sup>. O Pampa é caracterizado pela vegetação herbácea e arbustiva, possuindo diferentes composições de solo. Os principais tipos de uso da terra observados no Pampa são as pastagens/pecuária, a agricultura extensiva e a silvicultura (cultivo de *Eucalyptus* sp., *Pinus* sp., *Acacia* sp., entre outras), sendo que mais da metade da vegetação campestre já perdeu suas características originais em decorrência da atividade humana<sup>85</sup>. A cobertura vegetal dos diferentes biomas brasileiros permaneceu em grande parte preservada até a década de 1960, porém a partir da década de 1970 o uso da terra foi intensificado e a vegetação natural foi substancialmente substituída por pastagens plantadas<sup>86</sup>. A expansão de monoculturas como a soja tem descaracterizado o Pampa e reduzido sua biodiversidade, o que é preocupante considerando que a quantidade de Unidades de Conservação no Pampa é limitada (apenas 16)<sup>84</sup>.

Problemas relacionados à agricultura extensiva (monocultivo de soja, arroz, entre outros), como a erosão dos solos, contaminação por agroquímicos e redução da biodiversidade são comuns no RS<sup>87</sup>. Esses desequilíbrios ambientais que afetam o solo e a vegetação possivelmente têm um forte impacto sobre a distribuição e carga de ovos e larvas de parasitas no ambiente, porém este é um tópico ainda pouco estudado. Visto que a área de cultivo agrícola já supera as áreas de formação campestre no RS<sup>88</sup>, é fundamental considerar e investigar os impactos da produção agrícola sobre



o ciclo de desenvolvimento de geo-helmintos em solos sob influência da agricultura (Alguns questionamentos relevantes são: a contaminação aumenta ou diminuiu? Como a perda da biodiversidade afeta os geo-helmintos?).

A Mata Atlântica também enfrenta graves problemas em termos de conservação e regulamentação ambiental no RS<sup>89</sup>, sendo um bioma fortemente afetado pelo desmatamento<sup>90</sup>, processo que influencia a ocorrência das geo-helminthíases<sup>91</sup>. Por exemplo, um aumento dos casos de infecção por ancilostomídeos no Haiti foi atribuído ao desmatamento associado com enchentes, eventos que tornaram as condições do solo mais propícias ao desenvolvimento dos parasitas<sup>92</sup>. No entanto, as informações sobre como o desmatamento pode afetar a presença geo-helmintos no contexto Brasileiro são escassas.

Os biomas Pampa e Mata Atlântica contribuem para a manutenção de importantes serviços ecossistêmicos, como o armazenamento de estoques de carbono<sup>93</sup>. A criação de gado no Pampa é responsável pela emissão de uma importante quantidade de gases de efeito estufa, incluindo dióxido de carbono (CO<sub>2</sub>) e metano, especialmente sob práticas de manejo inadequadas e quando a quantidade de animais supera os limites comportados pelo bioma<sup>94</sup>. A produção de soja nos biomas Pampa e Mata Atlântica também contribui para a emissão atmosféricas de CO<sub>2</sub>, acelerando as mudanças climáticas, apesar das emissões de gases de efeito estufa decorrentes deste cultivo serem menores no RS em comparação com outros estados brasileiros onde o desmatamento é mais intenso<sup>95,96</sup>. As mudanças climáticas causarão alterações na distribuição geográfica das geo-helminthíases, visto que mudanças de temperatura e eventos climáticos extremos impactarão as condições de sobrevivência das estruturas parasitárias no solo. Migrações e outras questões demográficas e socioeconômicas decorrentes das mudanças climáticas também alterarão o cenário epidemiológico das geo-helminthíases nos próximos anos<sup>97,98</sup>.

## Conclusão

As porcentagens de geo-helmintos em diferentes amostras humanas e ambientais provenientes de estudos realizados no Rio Grande do Sul foram compiladas na **Tabela 2**, comparando-se os dados obtidos nos estudos mencionados ao longo desta revisão com os dados obtidos por Katz<sup>31</sup>. Apesar do RS ser um dos estados com as menores prevalências globais de geo-helminthíases no Brasil, essas taxas são bastante variadas quando populações de diferentes municípios e contextos socioambientais são comparadas. Similarmente, a contaminação ambiental por geo-helmintos varia nas diferentes paisagens urbanas dos municípios gaúchos. Fatores humanos, animais e ambientais contribuem para essas variações, indicando que estratégias alinhadas com a Saúde Única são fundamentais para o estudo, prevenção e controle das geo-helminthíases no RS (**Figuras 2 e 3**). Por

fim, ressalta-se que é necessário avaliar como impactos ambientais específicos afetam a distribuição de geo-helmintos nos biomas Pampa e Mata Atlântica.

**Tabela 2.** Percentagens de geo-helmintos em diferentes amostras humanas e ambientais provenientes de estudos realizados no Rio Grande do Sul. As percentagens citadas na tabela representam uma compilação dos valores obtidos dos estudos revisados neste artigo ('Revisão') e do inquérito coprológico nacional publicado por Katz<sup>31</sup>. nd = dados não disponíveis.

Geo-helminto	Fonte dos dados	Percentagens observadas (% mínima - % máxima)	
		Humanos	Ambiente (solo ou fezes coletadas no ambiente)
Ancilostomídeos	Revisão	3,4% - 42,1%	11,0% - 93,2%
	Katz <sup>31</sup>	0,00% - 0,67%	nd
<i>Ascaris lumbricoides</i>	Revisão	10,0% - 55,6%	3,1% - 10,2%
	Katz <sup>31</sup>	0,00% - 6,67%	nd
<i>Trichuris trichiura</i>	Revisão	2,0% - 36,0%	0,8% - 32,5%
	Katz <sup>31</sup>	0,00% - 13,33%	nd
<i>Toxocara</i> spp.	Revisão	nd	4,2% - 25,0%


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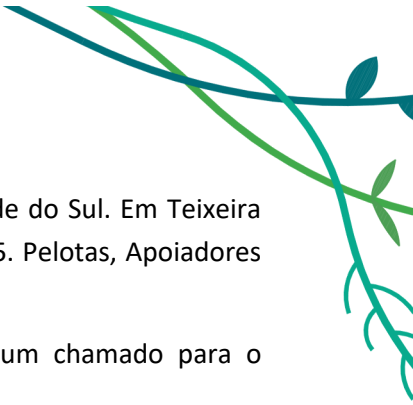
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### CAPITULO III

#### *Deficiência de ferro e infecção por geo-helminthos*

Esse capítulo apresenta um trabalho de revisão publicado na revista *Parasitology Research* sobre as conexões clássicas e negligenciadas entre a deficiência de ferro e a infecção por geo-helminthos.

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## CAPÍTULO IV

### *Geo-helminhos em amostras do Campus do Vale*

O trabalho que compõe este capítulo será submetido para a revista *Science in One Health* e se trata de um estudo piloto em que foram analisadas diversas amostras de solo em diversos pontos do Campus do Vale da Universidade Federal do Rio Grande do Sul.





Full length article

## Soil-transmitted helminths detected from environmental samples in a campus of southern Brazil



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### ABSTRACT

Soil harbours enormous biodiversity, essential for maintaining environmental and human health. However, soil can also be a reservoir of various parasitic pathogens, such as soil-transmitted helminths (STH). We evaluated the presence of STH (e.g., hookworms, roundworms and whipworms) in soil samples collected at twenty points within the perimeter of *Campus do Vale* (a university campus belonging to the Federal University of Rio Grande do Sul - UFRGS), during 2022 winter season. Considering the One Health perspective, human, animal and environment-related data from each sampling point were collected. All soil samples showed nematode larvae, representing natural components of soil biodiversity. Considering STH eggs, 35% ( $n = 7$ ) of soil samples showed hookworm eggs (e.g., from *Necator americanus* or *Ancylostoma duodenale*), 10% ( $n = 2$ ) showed roundworm (*Ascaris lumbricoides*) eggs, and 5% ( $n = 1$ ) showed whipworm (*Trichuris trichiura*-like) eggs. Of note, 10% of the sampling points showed the presence of rhabditiform hookworm larvae, 5% showed *Strongyloides stercoralis* rhabditiform larvae and 5% had the presence of filariform hookworm larvae, indicating a risk of human percutaneous infection. The significant people circulation in *Campus do Vale*, in association with other environment-related factors, help to explain the prevalence of STH observed in this study.

### 1. Introduction

Helminths are the most common human pathogenic parasites in developing countries. Helminth infections are major neglected tropical diseases considering disability-adjusted life years and other criteria [1]. Soil-transmitted helminths (STH) are enteric parasites with a biological cycle that includes an obligatory developmental stage in the soil, being capable of infecting humans and non-human animals through contact with contaminated soil. Roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) are considered the main human STH in different world regions [2].

Human infection by whipworms and roundworms usually occurs through the ingestion of mature eggs after contact with egg-contaminated soil or food. Third-stage hookworm larvae (L3 or filariform) infect humans through percutaneous invasion (*N. americanus* and

*A. duodenale*). Infection by *A. duodenale* can also occur by accidental oral ingestion, potentially resulting in Wakana syndrome [2,3]. *Strongyloides stercoralis* is also considered a member of the STH group; however, many health agencies do not include this parasite in their disease control programs. Therefore, *S. stercoralis* is often not considered in STH studies [4]. The population infected by *Strongyloides* is estimated at 30–100 million people worldwide [2]. Free-living *Strongyloides* filariform larvae found in the soil can infect humans through direct contact [5]. The soil can therefore serve as an ‘intermediate host’ during STH infection. STH eggs and larvae can develop and become infective in soils with proper conditions for the development and protection of the parasites [6].

Some hookworm species are classified as zoonotic parasites (e.g., *Ancylostoma braziliense*, *Ancylostoma caninum*) having animals (e.g., dogs, cats) as definitive hosts, but accidentally parasitizing humans. This zoonotic infection causes cutaneous larva migrans, an inflammatory disease triggered by the larvae migration through the human skin [7,8].

Abbreviations: STH, Soil-transmitted helminths.

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STH infections are important causes of impairment of physical growth and intellectual development in children, and can also trigger various health issues in adults, such as malnutrition, iron deficiency anemia and intestinal problems [3,9,10]. Eggs and larvae of STH are very adapted to tropical and subtropical climates, and both mono- and co-infection by STH are of great public health significance to low- and middle-income countries in Africa, Asia and Latin America, including Brazil [2,3,11].

The complex and porous structure of the soil harbours an enormous quantity and variety of animal species and microorganisms. The soil biodiversity varies depending on the content of organic material, chemicals, food supplies, water and sanitary condition of the environment. The soil biodiversity is also affected by anthropogenic activities, such as urbanization, agriculture and other types of land-use changes. Considering the enormous diversity of archaea, bacteria, protists, tardigrades, rotifers, nematodes, acari, collembolans, worms, macroarthropods and burrowing mammals found in soil, the current knowledge about soil biodiversity is limited. This occurs because (I) the identification of soil-associated species is difficult, (II) the small size of the organisms, (III) the lack of qualified professionals, among other reasons. This lack of knowledge refers to both organisms beneficial to humans and pathogenic species [12,13].

Nematodes comprise an important part of soil biodiversity, being considered good indicators of environmental quality. Soil nematodes include parasites that can infect plants, animals and humans (i.e. STH). The distribution of soil nematodes is affected by solar incidence, vegetation and chemical and physical characteristics of the soil [13]. STH are observed in several Brazilian regions and their prevalence is associated with the socioeconomic conditions of the population. Lack of access to safe water, sanitation and hygiene (WASH) and precarious housing conditions are important factors for environmental contamination and human infection by STH [14,15].

Katz [16] performed a national survey concerning STH prevalence in the Brazilian population, covering all Brazilian states. The Rio Grande do Sul state had a limited adherence to the survey (36.9%), compromising the reliability of the results (adherence to the survey ranged from 60% to 100% among the other states). Therefore, new regional studies are needed to analyze and update STH data for Rio Grande do Sul state, especially considering Porto Alegre city, the most populous city of the state. Moreover, Porto Alegre lost two places in Brazil's "sanitation ranking", now occupying position number 42. This unwelcome result may affect the occurrence of STH in the city. Of note, the sewage system network reaches only 91.3% of the Porto Alegre population, and the city only treats 82% of all the collected sewage ([17]; based on 2020 data). The lack of proper treatment of sewage produced in the city causes the dumping of organic carbon into Lake Guaíba, a water body of great environmental, economic and historical-cultural importance for the state, thus increasing levels of metals and phosphorus in the lake [18]. Taken together, these issues collaborate to increase the risks of STH infection for the population living in the Porto Alegre region.

Other factors make Porto Alegre an interesting city for parasitological studies. Porto Alegre is in an ecotone region. The diversity of geomorphological and biological structures observed in the transition between two biomes generates a mosaic of hills and plains covered by forests and grasslands [19]. *Campus do Vale* belongs to the Federal University of Rio Grande do Sul, one of the largest universities in Brazil, being located between the biomes Atlantic Forest (*Mata Atlântica*) and Pampa. *Campus do Vale* harbours the highest hill in the Porto Alegre city, called *Morro Santana*, which is an important and protected "green area". This ecological reserve is home to an enormous diversity of mammals [19] and about a quarter of the estimated grass diversity of the state [20]. Also, around 60 bird species were observed within *Campus do Vale* [21]. Many students, employees and members of the general population frequent *Campus do Vale* daily, and due to its biodiverse landscape, human interaction with insects, birds, primates and reptiles is common (authors' observation). Moreover, the presence of domestic animals, mainly dogs, circulating among people is a common observation in *Campus do Vale*. This occurs due to residential neighborhoods located

very close to the campus associated with the university community's habit of providing shelter and food for dogs.

Considering the scarcity of data concerning STH in the Porto Alegre region combined with the interesting ecological characteristics of *Campus do Vale*, the objective of this study was to evaluate the presence of STH eggs and larvae in soil samples at twenty sampling points distributed throughout the *Campus do Vale* (UFRGS), Porto Alegre, southern Brazil. Environmental, animal and human information and data were collected from each sampling site to help explain the results. This is a study developed under the One Health perspective [22,23] since it evaluated a complex public health problem considering human, animal and environmental aspects. Finally, we highlight that this is a pilot study for another larger study involving different sampling points located in the Porto Alegre region.

## 2. Materials and methods

### 2.1. Ethical statement

Soil sample collections were authorized by the *Sistema de Autorização e Informação em Biodiversidade* - SISBIO (*Instituto Chico Mendes de Conservação da Biodiversidade* - ICMBio, *Ministério do Meio Ambiente*, Brazil): SISBIO No. 82718-1. This study was also authorized by the Environment Department of UFRGS (DMALIC/SUINFRA - UFRGS). Biological samples collected during this study were also registered in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen (*Ministério do Meio Ambiente*, Brazil): registration codes ADC0B9C and A314A58.

### 2.2. Study area

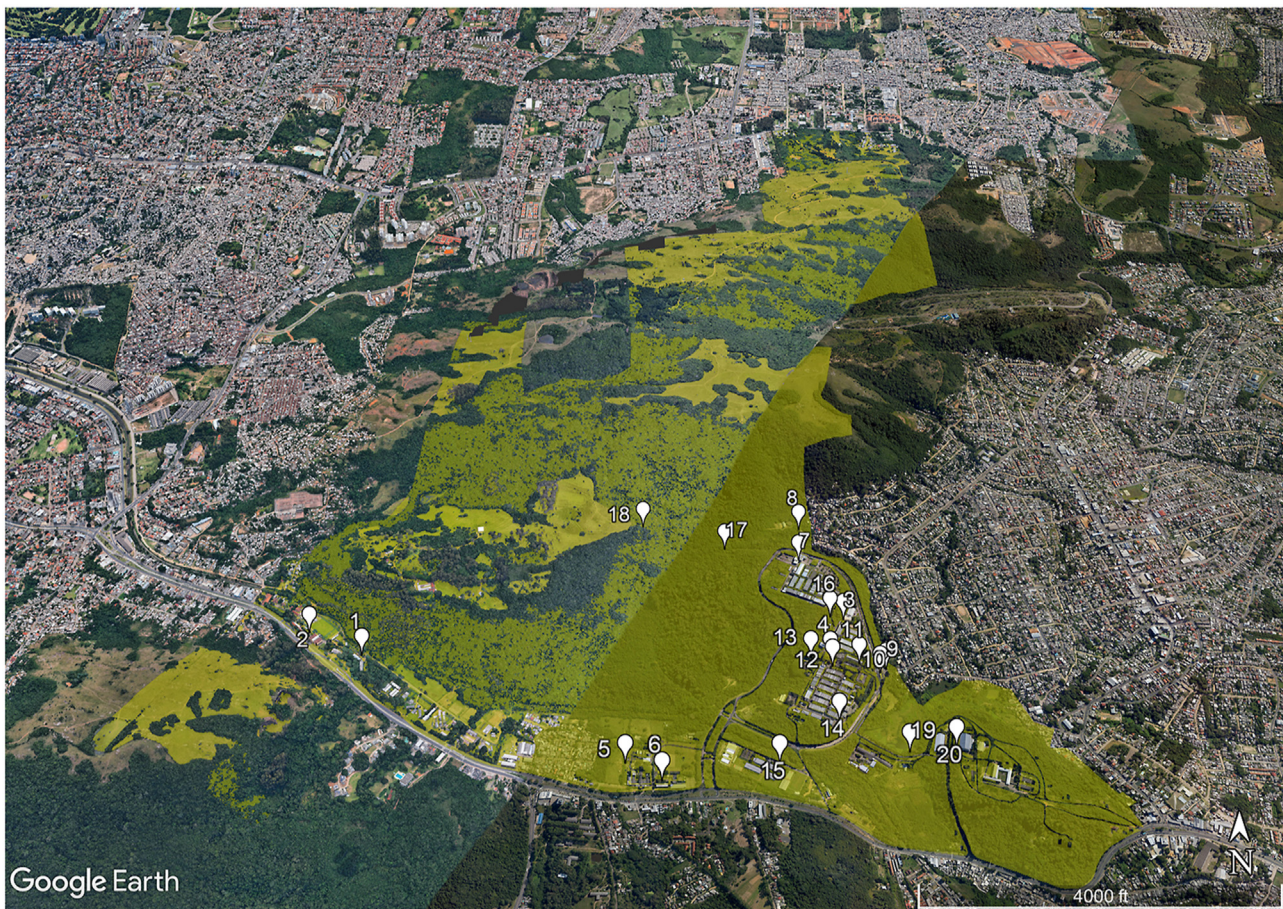
Soil samples were collected at twenty points of *Campus do Vale* (Fig 1 and Table 1), a university campus belonging to UFRGS and localized in Porto Alegre city, the capital of Rio Grande do Sul state, extreme south of Brazil. Porto Alegre has a territorial area of 495,390 km<sup>2</sup> [24] and an estimated population of 1,492,530 people [25]. Porto Alegre is an ecotone zone, composed of Atlantic Forest and Pampa (grasslands) biomes. The vegetation cover of Porto Alegre was heavily modified due to urbanization, agriculture and mining activities, among other types of anthropogenic land-use changes. Only 24.1% of the original vegetation cover remains in the region: 10.2% of grasslands and 13.9% of forest cover [26,27].

*Campus do Vale* has an extension of ~700 ha, with an estimated community of 30,000 people [28]. The campus includes more than 30 undergraduate courses, two university restaurants and six research centers. Some university units provide direct services to the community, such as the University's Veterinary Hospital and the Institute of Hydraulic Research (*Instituto de Pesquisas Hidráulicas* - IPH). Also, a dog shelter managed by an animal protection NGO operated until recently within the *Campus do Vale* (the NGO's activities ceased in March 2023). *Campus do Vale* encompasses part of *Dilúvio* Stream and the *Morro Santana* Ecological Reserve, which covers an area of 3,500,000 m<sup>2</sup>. This area also includes a region once occupied as a farmland, which was posteriorly acquired by the Brazilian government. *Campus do Vale* is an ecological reserve composed of natural landscapes with varied levels of regeneration. This ecological reserve exhibits a vegetation profile that includes rupestrian fields and regenerated subxerophytic and mesophyll forests [26].

### 2.3. Sampling method and evaluation of soil samples

Soil samples were collected and processed according to the Rugai's method [29] adapted for soil samples [30], during 2022 winter season. From each sampling point, we collected approximately 100 g of topsoil (up to 10 cm deep) using a clean garden shovel and sterile plastic tubes. The sampling points were selected with the objective of sampling areas of





**Fig. 1.** Sampling points distributed throughout *Campus do Vale* - UFRGS. Each number represents a sampling point. The name and characteristics of each sampling point are detailed in [Table 1](#).

*Campus do Vale* with different landscape characteristics concerning vegetation, presence of people and animals, and types of building ([Table 1](#)). The soil samples were placed into a polystyrene box and transported to the laboratory (Laboratory of Immunobiology and Immunogenetics - UFRGS). All samples were analyzed immediately after collection.

Each 100 g of freshly extracted soil were placed in four-layer packs of bandage gauze. The packs containing the soil samples were then placed in glass sedimentation cones filled with 45 °C water. The water temperature allows the capture of helminth larvae due to their thermotaxis and hydrotropism. The eggs of the parasites sediment by gravity, and larger soil components remain trapped in the four-layer packs of bandage gauze. After 1-h sedimentation, packs were removed from sedimentation cones and the material continued to settle for an additional 1 h. Afterwards, the sedimented material was removed from the bottom of the sedimentation cones with disposable Pasteur pipettes and transferred to microcentrifuge tubes, then centrifuged at 2000 rpm (380 G-force) for 2 min. Finally, an aliquot of the centrifuged material was placed under a microscope slide, stained with 2% Lugol, and covered with a coverslip. The slides were analyzed under the microscope by two trained microscopists under 100× and 400× magnification. The above-mentioned Rugai's method adapted for soil samples was based on Carvalho et al. [30], with minor adaptations. Studies performed by González y Cáceres et al. [31] and Carvalho et al. [32] were also considered during methodology standardization. This method allows the recovery of the following parasitic structures: eggs of *A. lumbricoides*, hookworms, *Taenia* sp., *Trichuris trichiura* and *Toxocara canis*, larvae of *S. stercoralis* and hookworms, and cysts of *Giardia lamblia*, *Entamoeba coli* and *E. histolytica* [30]. For this study, parasite cysts were not considered. Two slides were

analyzed for each sampling points (one slide per microscopists; 20 sampling points), totaling 40 slides in this study. The morphological identification of the parasites was based on the descriptions and recommendations of Neves [33], De Carli [34], Mariano et al. [35], and CDC [36].

#### 2.4. Collection of environment-related data

A form for the assessment of socio-environmental data and anthropic activity (available in Brazilian Portuguese as Supplementary Material) was filled at each sampling point. In this form, we recorded data concerning biotic and abiotic aspects that could influence the presence or absence of soil parasites, such as conditions of environmental sanitation, disposal of domestic and industrial waste, presence of regular housing, circulation and signs of animals and humans, among other environmental characteristics.

### 3. Results

[Table 1](#) shows detailed data concerning weather conditions, temperature, soil characteristic and vegetation of each sampling point. Soil samplings were performed during the winter season and due to this reason the temperatures at sampling days were mild (12.8 °C average) with half of sampling days showing rainy or cloudy conditions. Eleven sampling points (55%) showed ornamental vegetation; and organic soil was observed in most of the sampling points ( $n = 16$ , 80%).

[Table 2](#) shows results from environment-related data recorded at each sampling point. Most of the sites showed regular human dwellings/buildings and proper environmental sanitation ( $n = 15$ , 75%; for both

**Table 1**  
Geographic location, soil characteristics, vegetation and climatic conditions of each point recorded during the sampling.

Sampling point	Popular name <sup>a</sup> (translated from Portuguese)	Latitude	Longitude	Temp.	Climate condition	Soil characteristic	Vegetation
Point 1	University Restaurant no. 4, Agronomy	-30.071308	-51.138732	11 °C	Cloudy	Clayey	Ornamental
Point 2	Phylogenetic Garden, Agronomy	-30.070267	-51.140178	10 °C	Cloudy	Organic	Ornamental
Point 3	Department of Genetics	-30.069913	-51.119114	10 °C	Sunny	Organic	Ornamental
Point 4	Campus do Vale Center	-30.071547	-51.119821	10 °C	Sunny	Organic/Sandy	Ornamental
Point 5	Waste Center, Veterinary Medicine	-30.075629	-51.128026	14 °C	Sunny	Organic	Degraded natural grassland/shrub
Point 6	Academic Directory, Veterinary Medicine	-30.076296	-51.126637	14 °C	Sunny	Organic	Ornamental
Point 7	University Restaurant no. 6, Campus do Vale	-30.067304	-51.120744	13 °C	Sunny	Organic	Ornamental
Point 8	Patas Dadas	-30.065938	-51.120634	12 °C	Sunny	Organic	Degraded/artificial/reforested arboreal forest
Point 9	Neighborhood Entrance	-30.071983	-51.117632	13 °C	Rainy	Clayey	Degraded natural grassland/shrub
Point 10	Bus station	-30.072135	-51.117889	13 °C	Rainy	Organic	Ornamental
Point 11	Language and Arts Building	-30.071798	-51.118693	13 °C	Rainy	Organic	Ornamental
Point 12	Square between Language and Chemistry courses	-30.071890	-51.119771	14 °C	Rainy	Organic	Ornamental
Point 13	University Restaurant no. 3, Campus do Vale	-30.071513	-51.120597	14 °C	Rainy	Sandy	Degraded/artificial/reforested arboreal forest
Point 14	Microscopy Center	-30.074131	-51.119747	14 °C	Rainy	Organic	Ornamental
Point 15	Bus station, Aplicação school	-30.075950	-51.121351	15 °C	Rainy	Sandy	Ornamental
Point 16	Stairway	-30.069863	-51.119667	15 °C	Rainy	Organic	Degraded/artificial/reforested arboreal forest
Point 17	Santana Hill – entrance	-30.066905	-51.123875	15 °C	Sunny	Organic	Preserved arboreal forest
Point 18	Santana Hill–center	-30.066087	-51.127359	15 °C	Sunny	Organic	Preserved arboreal forest
Point 19	Dam Lake	-30.075259	-51.117172	11 °C	Sunny	Organic	Degraded/artificial/reforested arboreal forest
Point 20	Hydraulic Research Institute	-30.075140	-51.115393	11 °C	Sunny	Organic	Degraded/artificial/reforested arboreal forest

<sup>a</sup> Names commonly used by the University's community. Temp. = temperature (average).

**Table 2**  
Environment-related characteristics of each sampling point.

Sampling point	Human dwellings/buildings	Noise pollution	Environmental sanitation <sup>a</sup>	Domestic sewage disposal	Artificial mosquito larvae breeding sites	Domestic solid waste	Industrial/biological sewage disposal	Expected human circulation	Domestic animals (sightings or indications)	Synanthropic animals (sightings or indications)	Farm animals (sightings or indications)
1	Regular	Absent/light sounds	Proper	Absent	Present	Present	Absent	Frequent	Present: dog	Absent	Absent
2	Regular	Present	Proper	Absent	Absent	Present	Absent	Frequent	Absent	Absent	Absent
3	Regular	Present	Proper	Absent	Absent	Present	Absent	Frequent	Absent	Absent	Absent
4	Regular	Present	Proper	Absent	Absent	Absent	Absent	Frequent	Absent	Absent	Absent
5	Regular	Absent/light sounds	Proper	Absent	Present	Present	Absent	Frequent	Absent	Present: pigeon	Present: cattle, horse
6	Regular	Present	Proper	Absent	Absent	Present	Absent	Frequent	Absent	Absent	Absent
7	Regular	Present	Proper	Present	Absent	Present	Absent	Intense	Absent	Present: pigeon	Absent
8	Regular	Absent/light sounds	Proper	Absent	Absent	Present	Absent	Infrequent	Present: dog	Present: rodent	Absent
9	Regular	Present	Proper	Absent	Absent	Present	Absent	Frequent	Absent	Present: pigeon	Absent
10	Absent	Intense	Not necessary	Absent	Absent	Present	Absent	Intense	Absent	Absent	Absent
11	Regular	Present	Proper	Absent	Absent	Present	Absent	Intense	Absent	Absent	Absent
12	Regular	Present	Proper	Absent	Absent	Absent	Absent	Intense	Absent	Absent	Absent
13	Regular	Intense	Proper	Absent	Absent	Present	Absent	Intense	Absent	Present: rodent	Absent
14	Regular	Absent/light sounds	Proper	Absent	Absent	Absent	Absent	Not frequent	Absent	Absent	Absent
15	Regular	Absent/light sounds	Proper	Absent	Absent	Present	Absent	Not frequent	Absent	Absent	Absent
16	Absent	Absent/light sounds	Not necessary	Absent	Absent	Present	Absent	Frequent	Absent	Absent	Absent
17	Absent	Absent/light sounds	Not necessary	Absent	Absent	Present	Absent	Not frequent	Absent	Absent	Absent
18	Absent	Absent/light sounds	Not necessary	Absent	Absent	Absent	Absent	Not frequent	Absent	Absent	Absent
19	Absent	Present	Inappropriate	Present	Present	Present	Present	Not frequent	Absent	Present: wild rodents, urban birds	Absent
20	Regular	Absent/light sounds	Proper	Absent	Absent	Absent	Absent	Not frequent	Absent	Absent	Absent

<sup>a</sup> Environmental sanitation: Presence of treated water and sewage collection systems. Domestic solid waste (presence/absence) was considered as a separate category in this article.



**Table 3**  
Parasite larvae and eggs observed at each sampling point.

Larvae and eggs identified	Sampling points																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Microscopic nematode larvae	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hookworm (filariform) larvae				X																
Hookworm (rhabditiform) larvae				X	X															
<i>Strongyloides stercoralis</i> (rhabditiform) larvae			X																	
Hookworm eggs	X			X				X	X		X			X						X
<i>Ascaris lumbricoides</i> eggs										X						X				
<i>Trichuris trichiura</i> -like <sup>a</sup> eggs											X									

Each 'X' in the sampling point table cells indicates the presence of at least one of the parasitic structures (larvae or eggs) indicated in the column on the left. *Taenia* sp. and *Toxocara canis* eggs are not mentioned in the table because they were not observed in the analyses.

<sup>a</sup> Suggestive of *Trichuris trichiura* based on morphology.

criteria). However, inappropriate domestic solid waste disposal was observed in 15 (75%) sampling points. Due to the presence of *Dilúvio* Stream, which passes through the *Campus do Vale*, the disposal of both domestic and industrial sewage was observed at sampling point 19. Near the University Restaurant (sampling point 7), we also observed problems in the building plumbing, with domestic sewage partially licking into the environment. Domestic animals (dogs) were observed in only two sampling points. Observation or indicatives of synanthropic animals (especially pigeons and rodents) were recorded in 6 (30%) sampling points. The main indicator of synanthropic animals were rodent traps spread across the campus. Farm animals were observed only at the University's veterinary area (sampling point 5). Intense or frequent human presence was observed in 13 (65%) sampling points (Table 2).

Table 3 details the results of parasitological analyses for each sampling point. Microscopic nematode larvae were observed in all sampling points, indicating that the parasitological technique used in this study was effective in the recovery of larvae present in the soil. However, we stress that most of the observed larvae are not pathogenic for humans or animals but are part of the normal (healthy) soil biodiversity. Considering pathogenic larvae, rhabditiform hookworm larvae were observed in sampling points 4 and 5. Filariform (infective) hookworm larvae were observed only at sampling point 4. *S. stercoralis* rhabditiform larvae were observed at sampling point 3 (Table 3).

Considering parasite eggs, hookworm eggs were found in 7 sampling points (35%), followed by *A. lumbricoides* eggs, observed in 2 sampling points (10%). *Trichuris trichiura*-like eggs were observed only at sampling point 11 (Table 3), a place of intense human presence (Table 2). *Taenia* sp. and *T. canis* eggs were not observed at any sampling point.

#### 4. Discussion

Nematodes are one of the most diverse and widespread animal groups on the planet, participating in different levels of the soil food webs [37], and representing a significant part of the soil mass. Although some nematode species are parasitic on plants and animals, most species have a critical participation in the maintenance of soil health, thus providing food, increasing air and water quality, and regulating soil-related ecosystem services [13,38,39]. In regions constituted by grasslands, such as the Pampa biome, soil invertebrates contribute to plant species diversity and ecological succession [40]. Maintaining soil quality and nematode diversity are therefore critical for environmental and human health [12]. In this study, all soil samples showed an abundance of microscopic nematodes, indicating a rich soil biodiversity. This finding is probably due, at least in part, to the organic conditions of the soil found in most of the samples analyzed. Of note, morphological differentiation of pathogenic parasites (i.e., STH) from non-pathogenic nematodes in soil samples must be performed carefully due to the great abundance and diversity of nematodes found in soil. In the context of human parasitology, microscopists used to evaluate stool samples should be aware concerning the peculiarities and biodiversity of soil when analyzing STH in environmental samples. This careful approach is necessary in order to

avoid misinterpretation of the presence of non-pathogenic nematodes, natural components of the soil biodiversity. Nevertheless, although most of the nematodes observed in the study were non-pathogenic components of soil, a small portion of the nematode larvae were part of the human pathogenic STH group.

STH are distributed throughout the Brazilian territory [16] and their prevalence is associated with socioeconomic conditions since lack of environmental sanitation, poor hygienic conditions, ingestion of non-potable water, walking barefoot and consumption of contaminated food are some factors that favor the occurrence of STH eggs and larvae in the soil and, consequently, human infection [8]. Adult stages of major STH inhabit the human gastrointestinal tract, where they can stay for months and even years. Hookworms and *Ascaris* sp. are usually found in the small intestine, whereas *T. trichuria* are found in the cecum, colon and rectum, depending on infection level [9]. Clinical aspects and morbidity are commonly associated with infection intensity [41], and STH eggs are passed through the feces to the external environment [2,42]. Eggs and larvae of STH can thrive in warm and moist soil for several months [2]. Hookworm larvae are free living organisms in the soil. The rhabditiform larva is the first larval stage, and it becomes infective when it reaches its third (filariform) larval stage [34].

In this study, we observed in the soil samples larvae of hookworms and *S. stercoralis*, eggs of *A. lumbricoides*, and suggestive eggs of *T. trichiura*. The presence of infective larvae demonstrates that the studied environment is indeed conducive to the survival and development of STH, even during the winter season, representing a risk for human infection. Our results are similar to other studies carried out in schools and university campi localized in southern Brazil (reviewed by Ziliotto et al. [43]), despite indicating that *Campus do Vale* is a less contaminated environment than public urban areas in Porto Alegre. In fact, a previous study performed by Vargas et al. [44] analyzed the presence of different parasitic structures in public squares and parks of Porto Alegre city; and 100% of the analyzed places were contaminated with some parasite [44].

The intense presence of people in *Campus do Vale* (~30,000 individuals) [28] helps to explain the occurrence of STH in the study area, even with proper environmental sanitation conditions, as observed in most sampling points. *Campus do Vale* is frequented by many professors, students, employees and members of the general community, including children, once UFRGS provides a variety of services to the community (e.g., veterinary services, language courses, food services), which facilitates the direct and indirect deposition of STH eggs in soil of the study area. Of note, a residential neighborhood with precarious housing conditions is located right next to the *Campus do Vale*, which also helps to explain the presence of STH in the area even under adequate sanitation conditions. It is essential to stress that parasite eggs can be transported between different locations by domestic animals, the soles of shoes, and attached to invertebrates, among other ways [45–47]. Public transport can also contribute to the dissemination of eggs, larvae and cysts of intestinal parasites since parasitic structures can be attached and transported on the buses' surface, for example [48]. Our study also

highlighted issues that need to be improved in *Campus do Vale*, such as the disposal of waste observed at sampling point 19 (Dam Lake) and the frequent presence of domestic waste scattered throughout the campus. In brief, the soil of *Campus do Vale* is contaminated with STH and may pose a risk of human contamination, similarly to the risks offered by soils from parks and public squares. However, it is important to emphasize that this contamination risk depends on inadequate hygiene practices that lead to the accidental ingestion of soil and associated STH eggs, such as not washing hands after handling soil.

No *Taenia* sp. eggs were observed in the soil samples, which was an expected result. Although veterinary data confirm the circulation of *T. solium* and *T. saginata* in livestock from different regions of Rio Grande do Sul [49,50], both taeniasis and human cysticercosis are not major problems in the state. Although epidemiological data on human taeniasis and cysticercosis in Rio Grande Sul are scarce, the presence of *Taenia* eggs in soil samples is not common in public areas in Brazil. Considering seven Brazilian studies reviewed by Araújo et al. [51], only one study from the Northeast Region [52] reported *Taenia* eggs in soil samples.

Furthermore, no *T. canis* eggs were observed in the soil samples in this study. Unlike *Taenia* sp., this was a surprising result since *T. canis* eggs were quite common in other similar studies (reviewed by Ziliotto et al. [43]). *T. canis* is a zoonotic parasite associated with the presence of dog feces [8]. Although signs of domestic animals were seen with some frequency in our study, the incidence of these animals was lower than expected (compared to authors' observations/experience from previous years). This is potentially due to the drop in the circulation of people at the university during the COVID pandemic (and consequent decrease in food availability) and improvements in the dog shelter present at *Campus do Vale* before COVID pandemic. However, the absence of *T. canis* eggs in our samples can also be ascertained to methodological limitations. Although the morphological analysis based on microscopy is classically considered the gold standard in parasitology studies, the particular method we used may not be as accurate as molecular methods for *T. canis* eggs, representing a limitation of this study. As mentioned earlier, this is a pilot study that will serve as the basis for a larger study to be carried out with soil samples from various regions of Porto Alegre. For this new study, we therefore will include molecular analyses for the detection of *T. canis* DNA in the samples, along with the microscopy analyses.

Finally, the analysis of soil samples is an important approach for preventing possible parasite-related infection cases, as it suggests the infection risk to which individuals or populations are exposed to. Also, it contributes to targeting parasite infection control programs to areas with important levels of parasite contamination [53]. Considering the importance of medical and socioeconomic effects of neglected tropical diseases on Brazil population [54], limited information regarding STH infections are available for Rio Grande do Sul state. This information is essential for control and prevention programs focused on southern Brazil [43]. In this context, this study provides new and relevant data concerning environmental contamination by STH in Porto Alegre city.

## 5. Conclusions

This study reports the occurrence of larvae of hookworms and *S. stercoralis*, eggs of *A. lumbricoides*, and suggestive eggs of *T. trichiura* in soil samples of *Campus do Vale*, Porto Alegre, Brazil. The intense presence of people in the study area in association with other environment-related factors help to explain the prevalence of STH observed in this study. Finally, this study contributes for the understanding of STH distribution in a biodiverse and environmental complex area in southern Brazil.

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## CRedit author statement

Marina Ziliotto: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Project administration. Joel Henrique Ellwanger: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Review & Editing, Visualization, Project administration, Supervision. José Artur Bogo Chies: Investigation, Writing - Review & Editing, Supervision, Funding acquisition.

## Conflicts of interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soh.2023.100016>.

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## CAPÍTULO V

### *Geo-helminhos em amostras de Porto Alegre*

O trabalho apresentado neste capítulo se trata de um artigo em preparação que será submetido para a revista *Eco-Environment & Health* e traz os resultados das análises de microscopia e moleculares que avaliam a presença de geo-helminhos, *Toxoplasma gondii* e *Toxocara canis* e *T. cati* em amostras de diferentes pontos da cidade de Porto Alegre.



## Article

# Soil-Transmitted Parasites and Non-Pathogenic Nematodes in Different Regions of Porto Alegre City, Brazil: A Comparison between Winter and Summer

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**Abstract:** We assessed the prevalence of soil-associated parasites and non-pathogenic nematodes in eight public areas of Porto Alegre (Rio Grande do Sul state, southern Brazil), the most populous city in Rio Grande do Sul. Soil samplings were carried out during the winter of 2022 and summer of 2023: A total of 80 samples were collected in winter and 80 in summer (ten samples from each sampling site per season), totaling 160 soil samples. The frequency of microscopic non-pathogenic nematode larvae was significantly higher ( $p = 0.048$ ) in winter (93.75%) than in summer (82.50%). Considering the pooled data from winter and summer ( $n = 160$ ) for human pathogenic parasites, the following frequencies were observed (using microscopy analysis): hookworm (filariform) larvae (1.25%), hookworm (rhabditiform) larvae (11.25%), *Strongyloides* spp. (filariform) larvae (0.63%), *Strongyloides* spp. (rhabditiform) larvae (2.5%), hookworm eggs (10.63%), *Ascaris* spp. eggs (10.00%), and *Trichuris* spp. eggs (1.25%). Hookworm (rhabditiform) larvae were the most frequent parasitic structures (15.00%) in winter, and *A. lumbricoides* eggs were the most frequent parasitic structures (8.75%) in summer. No statistically significant difference was observed in the frequency of pathogenic parasites between the seasons ( $p > 0.05$ ). *Toxoplasma gondii* DNA was assessed, but all soil samples tested negative in molecular analysis. Our results indicate that soil from many regions of Porto Alegre shows a high prevalence of soil-transmitted helminths, indicating the need for improvements in social conditions and environmental sanitation in the city. Our study also suggests that climate change may affect soil biodiversity, potentially harming non-pathogenic nematodes and favoring human pathogenic parasites.

**Keywords:** Brazil; climate; soil-transmitted helminths; environmental sanitation; one health; parasites



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## 1. Introduction

Parasites are organisms found in virtually all ecosystems, and parasitic infections can influence various aspects of the host's life, including health conditions and behavior [1,2]. Also, the effects of some parasitic infections on human health can trigger major economic losses. Neglected tropical diseases (NTD), including those caused by parasites, are more prevalent in the world's poorest and marginalized populations than in developed countries. In this context, NTD persists amongst these populations due to a range of socioeconomic and environmental issues. Vulnerable and poor populations have limited social and economic power to call attention to their health problems, facing a greater disease burden than other more privileged populations [3]. Moreover, anthropogenic environmental changes (e.g., deforestation, biodiversity loss, pollution, unplanned urbanization, and climate change) facilitate human exposure to several emerging and neglected pathogens, including parasites, which mainly affect populations with socioeconomic vulnerability [3,4].

Soil-transmitted helminths (STH) are a group of parasites with an obligate life cycle in the soil. Considering their distribution and public health importance, the main STH

are hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*), and whipworms (*Trichuris trichiura*). *Strongyloide stercoralis* is also an important STH species in countries such as Brazil. STH transmission occurs mainly through contact with feces or soil contaminated with parasite eggs, and the prevalence of STH infections is related to socioeconomic and environmental aspects, such as family income, schooling level, and environmental sanitation [5–7].

STH is part of the NTD group and is endemic to at least 120 countries [8]. STH infections cause high morbidity and are associated with impaired intellectual development and growth in children, iron-deficiency anemia, intestinal problems, and malnutrition [7,9]. In 2019, these parasitic infections were estimated to be responsible for the loss of 1.9 million disability-adjusted life years (DALYs) globally [10].

In addition to STH, other parasites with zoonotic potential are present in the soil, such as *Toxoplasma gondii* [11], a cosmopolitan obligate intracellular protozoan that parasitizes practically any warm-blooded animal. Humans infected with *T. gondii* can develop toxoplasmosis, which is a zoonosis with a wide geographical distribution. The prevalence of toxoplasmosis is influenced by human cultural patterns (e.g., food and hygiene habits), socioeconomic conditions, and access to environmental sanitation [12].

Inadequate water, sanitation, and hygiene (WASH) are critical drivers of the parasitic disease burden, especially among children from low-income and middle-income countries. The disease burden linked to inadequate WASH scores can be significantly reduced (potentially eliminated) by improving WASH indicators [13], thereby reducing human exposure to unhealthy environments [14]. Preventive chemotherapy and improvements in WASH scores are the main recognized measures for decreasing STH infections [15]. Although the morbidity associated with STH infections is controlled by preventive chemotherapy, the interruption of STH transmission cycles will only be effective when populations have access to adequate sanitation systems [16]. Sanitation, safe water, and hygiene are health-promoting components with different characteristics [17], but often approached each other because they bring complementary benefits. Sanitation is currently understood as a way of promoting health, quality of life, well-being, and sustainable development [18].

Undeniably, the expansion of the sanitation system in Brazil has had a positive impact on public health. Until 1930, infectious and parasitic diseases were responsible for 45% of the deaths in Brazil. This percentage was reduced to less than 5% in 2010, largely due to the expansion of the sanitation structures in the country [18]. However, this progress is both slow and insufficient. For example, Brazil occupies the 83rd position in the global sanitation ranking. In 2020, only 48.7% of the Brazilian population had access to safely managed sanitation, an increase of approximately only 13% compared to the data from 2000 [19]. Access to safe sanitation for less than 50% of the population is a serious and problematic situation for health, social, and environmental reasons.

Porto Alegre city, the capital of Rio Grande do Sul state (southern Brazil), showed important advances concerning environmental sanitation until 2015, when investments on sewage treatment stagnated. The lack of continuous investments caused the city to lose its position in the Sanitation Ranking of *Instituto Trata Brasil*, which evaluates the evolution of water, sewage, investment, and water loss indicators in the largest Brazilian cities. Porto Alegre was ranked 24th position in the 2017 ranking to the 43rd position in the 2022 ranking [20,21], which represents a significant setback.

Considering (i) the importance of access to adequate sanitation for the prevention of infections by STH and other parasites, and (ii) the lack of updated information on the environmental prevalence of these parasites in Porto Alegre, in this study we evaluated the presence of soil-transmitted parasites in eight regions of Porto Alegre. For comparison purposes, this evaluation was performed in both winter and at summer, using microscopy and molecular techniques. We also collected socio-environmental information (considering human, animal, and environmental factors) from each study site to help explain our findings from the One Health perspective.

## 2. Results

### 2.1. Characteristics of the Sampling Sites

Table 1 details geospatial information, temperature, weather conditions, and soil characteristics at the time of samplings for each study site in both seasons. In winter, the temperature varied between 13 °C and 21 °C (17 °C average), with cloudy weather being recorded in most sampling days. During summer, the average temperature was 26 °C, and most days presented sunny weather. The soil samples showed mostly organic composition.

**Table 1.** Basic information of each sampling site (winter and summer seasons).

Sampling Sites	Popular Name <sup>1</sup>	Latitude	Longitude	Temperature (Average), Winter	Temperature (Average), Summer	Weather Condition, Winter	Weather Condition, Summer	Soil Characteristic
Site 1	Alfândega Square	−30.028917	−51.230997	13 °C	26 °C	Cloudy	Cloudy	Organic; Sandy
Site 2	Mascarenhas de Moraes Park	−29.981681	−51.186776	20 °C	28 °C	Cloudy	Sunny	Organic; Sandy; Clayey
Site 3	Miguel Anibal Square	−30.012382	−51.132493	13 °C	25 °C	Cloudy	Cloudy	Organic; Sandy; Clayey
Site 4	Chico Mendes Park	−30.026889	−51.112667	21 °C	22 °C	Cloudy	Cloudy	Organic; Sandy; Clayey
Site 5	Doctor Jurandy Barcellos da Silva Square	−30.076251	−51.219721	20 °C	28 °C	Cloudy	Sunny	Organic; Sandy; Clayey
Site 6	Ipanema Beach <sup>2</sup>	−30.133273	−51.237503	18 °C	28 °C	Cloudy	Sunny	Organic; Sandy
Site 7	Saint'Hilaire Park	−30.097943	−51.113585	15 °C	27 °C	Cloudy	Sunny	Organic; Clayey
Site 8	Espigão Square	−30.241057	−51.086015	20 °C	25 °C	Sunny	Sunny	Organic; Sandy; Clayey

<sup>1</sup> Translated from Brazilian Portuguese popular names. <sup>2</sup> Freshwater beach.

Table 2 shows information concerning to socio-environmental characteristics of each sampling site (compiling data from both seasons). Considering vegetation, except the Saint'Hilaire Park (site 7), which has a well-preserved native vegetation, and Ipanema Beach (site 6), which is on the edge of Lake Guaíba, all other places have a predominance of ornamental vegetation, mixing native and exotic species. Three locations had inadequate environmental sanitation (sites 2, 6 and 7). The Mascarenhas de Moraes Park (site 2) had evident untreated sewage disposal in a stream present in the urban perimeter. In all sites, improper disposal of domestic waste was observed. We highlight Espigão Square (site 8), where incorrect disposal of hospital waste (many used syringes) was observed, in addition to an alarming amount of garbage disposed throughout the region. In almost all sites ( $n = 7$ ; 87.5%), incorrectly discarded garbage formed potential artificial breeding ground for the development of mosquito larvae. Also, the presence of dogs was observed, directly or indirectly, in all sites. Considering synanthropic animals, the main indicators were pigeon feces, observed in six sites (75%). We also observed human feces at three sites (37.8%) (Table 2).

**Table 2.** Socio-environment-related characteristics of each sampling site (considering data collected during winter and summer seasons together).

Sampling Sites	Local Vegetation	Human Dwellings/Buildings	Noise Pollution	Environmental Sanitation	Domestic Sewage Disposal	Artificial Mosquito Larvae Breeding Grounds	Domestic Solid Waste	Industrial/Biological Sewage Disposal	Expected Human Circulation	Domestic and Farm Animals <sup>1</sup>	Synanthropic Animals <sup>1</sup>	Human Feces
Site 1	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Intense	Present, dogs	Present, pigeons and rodents	Present
Site 2	Ornamental	Regular	Absent/light sounds	Insufficient	Present	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Present
Site 3	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Infrequent	Present, dogs	Present, pigeons	Absent
Site 4	Ornamental	Regular	Absent/light sounds	Proper	Absent	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Absent
Site 5	Ornamental	Regular	Present	Proper	Absent	Present	Present	Absent	Frequent	Present, dogs and cats	Present, pigeons	Present
Site 6	Naturally absent	Regular	Present	Insufficient	Present	Present	Present	Absent	Frequent	Present, dogs	Present, pigeons	Absent
Site 7	Natural grasslands/shrubs and preserved arboreal forest	Irregular	Absent/light sounds	Insufficient	Present	Absent	Present	Absent	Infrequent	Present, dogs and horses	Absent	Absent
Site 8	Ornamental and degraded arboreal forest	Regular	Present	Proper	Absent	Present	Present	Absent	Infrequent	Present, dogs	Absent	Absent

<sup>1</sup> Direct observation or signs indicating the presence of these animals.

## 2.2. Frequency of Parasite/Nematode-Positive Sites

Table 3 details results obtained with microscopy analyses. Each + mark in the table indicates the presence of at least one positive sample for non-pathogenic nematodes and pathogenic parasite (larvae or eggs) at the sampling site. Nematode larvae were observed in all sampling sites, both in summer and winter seasons. Considering pooled results from winter and summer (number of + sites in both seasons), we observed hookworm eggs in seven (87.5%) study sites, *Ascaris* spp. eggs in six (75%) sites, and *Trichuris* spp. eggs in one (12.5%) site. We found hookworm rhabditiform larvae in 100% of the study sites, and hookworm filariform larvae (infective form) in two (25%) sites. We also observed *Strongyloides* spp. rhabditiform larvae in three (37.5%) sites, and filariform larvae in one (12.5%) site (combined winter + summer data).

Considering only the positive results obtained during winter, we observed hookworm filariform larvae at one (12.5%) sampling site, and hookworm rhabditiform larvae at seven (87.5%) sampling sites. Also, during the winter, *Strongyloides* spp. filariform larvae were observed at site 4, and *Strongyloides* spp. rhabditiform larvae at sites 1 and 2. Regarding parasite eggs, hookworm eggs were observed at almost all sites ( $n = 7$ ; 87.5%), *A. lumbricoides* eggs at five sites (62.5%), and *T. trichiura*-like eggs were observed at only one site (12.5%) during winter (Table 3).

Also considering the number of positive sites, but now looking at summer data, hookworm filariform larvae was observed at one sampling site (12.5%), and hookworm rhabditiform larvae was observed at five sampling sites (62.5%). *Strongyloides* spp. filariform larvae were not observed during the summer, whereas *Strongyloides* spp. rhabditiform larvae were observed at two sites (25%). Hookworm eggs were observed at five sites (62.5%) and *A. lumbricoides* eggs were observed at six sites (75%). *Trichuris* spp. eggs were not observed during the summer (Table 3).

**Table 3.** Microscopic non-pathogenic nematodes and pathogenic parasite larvae and eggs observed at each sampling site during winter and summer seasons.

Observed Parasite Larvae and Eggs	Sampling Sites															
	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8	
	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)	Winter (n = 10)	Summer (n = 10)
Non-pathogenic nematode larvae <sup>1</sup>	+(10)	+(9)	+(9)	+(8)	+(10)	+(9)	+(10)	+(10)	+(9)	+(9)	+(9)	+(3)	+(9)	+(8)	+(9)	+(10)
Hookworm (filariform) larvae								+(1)					+(1)			
Hookworm (rhabditiform) larvae		+(1)	+(1)		+(1)	+(1)	+(2)	+(2)	+(2)		+(2)	+(1)	+(1)		+(3)	+(1)
<i>Strongyloides</i> spp. (filariform) larvae							+(1)									
<i>Strongyloides</i> spp. (rhabditiform) larvae	+(1)	+(1)	+(1)							+(1)						
Hookworm eggs <sup>2</sup>	+(1)		+(1)		+(1)	+(2)	+(2)	+(1)	+(1)	+(1)	+(2)	+(1)	+(3)	+(1)		
<i>Ascaris</i> spp. eggs	+(1)	+(1)		+(1)	+(1)	+(1)	+(2)	+(2)	+(2)	+(1)	+(3)	+(1)				
<i>Trichuris</i> spp. eggs	+(2)															

Each + indicates the presence of at least one positive sample for non-pathogenic nematodes and pathogenic parasite (larvae or eggs) at the sampling site. The number of positive samples at each site is shown in parentheses. <sup>1</sup> Non-pathogenic to humans. Components of natural soil biodiversity. <sup>2</sup> Potentially *Necator* spp. and *Ancylostoma* spp. eggs.

### 2.3. Frequency of Microscopic Non-Pathogenic Nematodes and Pathogenic Parasites between Seasons

Table 4 shows the frequency of positive samples based on microscopic analysis, considering pooled data from winter and summer, as well as data from each season separately. The frequencies of non-pathogenic nematode larvae were significantly different between seasons ( $p = 0.048$ ), with a higher frequency in winter (93.75%) than in summer (82.50%).

**Table 4.** Comparison of the proportions of positive samples between winter and summer for each parasite.

Observed Parasite Larvae and Eggs	Winter + Summer ( $n = 160$ )	Winter ( $n = 80$ )	Summer ( $n = 80$ )	Fisher's $p$ -Value (Winter versus Summer)
Microscopic nematode larvae <sup>1</sup>	141 (88.13%)	75 (93.75%)	66 (82.50%)	<b>0.048</b>
Hookworm (filariform) larvae	2 (1.25%)	1 (1.25%)	1 (1.25%)	1.000
Hookworm (rhabditiform) larvae	18 (11.25%)	12 (15.00%)	6 (7.50%)	0.210
<i>Strongyloides</i> spp. (filariform) larvae	1 (0.63%)	1 (1.25%)	0 (0%)	1.000
<i>Strongyloides</i> spp. (rhabditiform) larvae	4 (2.5%)	2 (2.50%)	2 (2.50%)	1.000
Hookworm eggs <sup>2</sup>	17 (10.63%)	11 (13.75%)	6 (7.50%)	0.305
<i>Ascaris</i> spp. eggs	16 (10.00%)	9 (11.25%)	7 (8.75%)	0.793
<i>Trichuris</i> spp. eggs	2 (1.25%)	2 (2.50%)	0 (0%)	0.497

<sup>1</sup> Non-pathogenic for humans. Natural components of soil biodiversity. <sup>2</sup> Potentially *Necator* spp. and *Ancylostoma* spp. eggs. Statistically significant  $p$ -value is shown in bold.

Considering pathogenic parasites, pooled data from winter and summer showed that the most frequent parasitic structures were hookworm (rhabditiform) larvae (11.25%), hookworm eggs (10.63%), and *Ascaris* spp. eggs (10.00%) (Table 4). Hookworm (rhabditiform) larvae were the most frequent parasitic structures (15.00%) during winter, and *Ascaris* spp. eggs were the most frequent parasitic structures (8.75%) during summer. No statistical difference was observed in the frequency of pathogenic parasites between the seasons ( $p > 0.05$  in all tests; Table 4).

### 2.4. Molecular Analysis

According to the results obtained from the PCR with specific primers, none of the 64 soil samples analyzed showed bands indicative of the presence of *T. gondii* DNA. Notably, positive and negative controls satisfactorily ensured the quality of PCR reactions.

## 3. Discussion

The United Nations has established water supply and sanitation as one of the Sustainable Development Goals [22], considering that environmental sanitation promotes human and environmental health, and controls multiple infectious and parasitic diseases [23,24]. The dynamics of WASH-related diseases are also affected by different features, such as population density, housing issues, concentration of pathogenic agents to which an individual is exposed, genetic and immune human susceptibility, and other factors [7,25,26]. The transmission of parasites in urban areas also depends on biological and socioeconomic factors. For instance, the prevalence of pathogenic parasites found in soil samples is strongly influenced by sewage treatment systems in cities [24]. Raw sewage can contain a large load of parasite eggs, contaminating soil and water where the sewage is improperly discharged. In addition, inequalities and inadequate housing can facilitate the contamination of the population in two ways: (i) irregular housing may be close to streams or water contaminated by untreated raw sewage, and (ii) lack of access to treated sewage is more common in slums and irregular housing, facilitating the contact of vulnerable populations with contaminated water and soil [27,28].

In this study, we evaluated the public areas in eight main regions of Porto Alegre city. Almost all sampling sites had adequate sanitation infrastructure, although evidence



of irregular garbage disposal was observed in all studied areas. As a matter of fact, the presence of an adequate sanitation infrastructure cannot, per se, totally prevent the occurrence of other situations that may compromise the environment and affect public health. One example is the presence of human and animal feces in the environment, even in areas with adequate sanitation. The presence of irregular garbage disposal and feces is not surprising, considering that the studied areas encompassed large public green areas, in the city, with a high circulation of people and animals. Nevertheless, we also observed the disposal of untreated sewage in the Mascarenhas de Moraes Park, evidencing the insufficient environmental sanitation in Porto Alegre.

The presence of non-pathogenic nematode larvae (components of the soil's natural biodiversity) [29,30] in 100% of the analyzed sites can be regarded as methodological control. In this sense, one can argue that the methodology was well executed since Rugai's method adapted for soil aims to recover live larvae [31]. Nevertheless, we observed a lower frequency of samples positive for non-pathogenic nematode larvae during summer than during winter. The abundance and structure of nematode population are sensitive to changes in the environment and season-related conditions [32,33]. Specifically, soil characteristics (moisture and temperature) are major factors that influence nematode populations, along with food availability, interactions with other organisms, and physical-chemical conditions of the environment [32]. Therefore, the differences between analyses carried out in the Rio Grande do Sul state may also be related to the climate and soil diversity of the state, a very biodiverse region covered by two biomes (Pampa and Atlantic Forest), and an extensive marine coast [7].

These results could also be related to the period of intense drought that Rio Grande do Sul experienced in the months prior to our summer samplings due to the occurrence of the oceanic and atmospheric phenomenon known as "La Niña", allied to the climatic consequences associated to Amazon deforestation [34,35]. These conditions may have contributed to a reduction in the population of microscopic soil nematodes. From a broader perspective this result clearly highlights that climate change will indeed have an important impact on soil biodiversity and nematode communities, as suggested elsewhere [36–39].

Considering the main STH, our results concerning microscopy analyzes (frequencies detailed in Table 4) are in line with other studies carried out in the Rio Grande do Sul state (reviewed by Ziliotto et al. [7]). Based on data compiled from studies performed in the state, hookworms were found in a range of 11.0% to 93.2% of environmental samples, *A. lumbricoides* in a range of 3.1% to 10.2%, and *T. trichiura* in a range of 0.8% to 32.5% of environmental samples [7]. Also, our observations of filariform and rhabditiform *Strongyloides* spp. larvae are not surprising since strongyloidiasis is endemic in Brazil [5].

The fact that we did not observe differences in STH prevalence between seasons (Table 4), although non-pathogenic nematode occurrence was affected (as discussed above), suggests that pathogenic soil-parasites are more resilient to climatic variations than are non-pathogenic soil fauna. This result is in line with studies suggesting that climate change contributes to biodiversity loss, favoring pathogenic organisms that are more resilient to environmental changes [4]. Also, an increase in temperatures and precipitations may favor the STH development in the soil [40].

To the best of our knowledge, the last study carried out in Porto Alegre with the objective of analyzing the presence of parasitic structures in soil samples was published in 2013 [41]. In this study, 100% of the analyzed sites had contaminated soil, with 40.8% of them showing parasitic structures. The frequency of *A. lumbricoides* eggs was 10.2%, and the frequency of *T. trichiura* eggs was 4.4% [41], both frequencies similar to those found in our study (Table 4). This suggests that parasite contamination in Porto Alegre's soil has remained stable over the last 10 years.

Another parasite of medical interest found in the soil by Vargas et al. [41] was *Toxocara* spp., with a frequency of 4.2%. In contrast, no *Toxocara* eggs were observed in our samples. Concerning *T. gondii*, no DNA positivity was observed for *T. gondii* in the samples. Considering the high incidence of *Toxocara* spp. and *T. gondii* observed in other studies [42–44],



we expected to find *Toxocara* spp. and *T. gondii* in our samples. We believe that the small amount of soil ( $0.2 \pm 0.05$  g) used in the DNA extraction kit could have limited the detection capacity of *T. gondii* DNA, potentially explaining our results. The technique used by Vargas et al. [41] includes a flotation process, which is commonly used to recover *Toxocara* eggs in the soil, due to the characteristics of the egg [45]. However, Rugai's method does not include this process. Differences between the microscopy-based parasitological methods used by us and those used by Vargas et al. [41] potentially explain the discrepancy in the results concerning *Toxocara* eggs.

In other Latin American countries, various techniques have been used to assess the presence of STH in soil samples [46–49]. For example, using washing, sedimentation, and flotation techniques, Falcone et al. [49] found the presence of parasites in 31% of soil samples from Argentina, including *A. lumbricoides* (1.1%) and hookworms (5.6%) [49]. Also, in the subtropical northern border of Argentina, Riveiro et al. [48] evaluated environmental contamination by parasites using a modified version of the Shurtleff and Averre method. The authors found that 37.5% of the soil samples were contaminated with parasites, with hookworms being the most prevalent parasite (28.8%) parasite. *Toxocara* spp. (6%), *Trichuris* spp. (3.8%) and *Ascaris* spp. (1.1%) were also found [48].

Important advances in the direction of sanitation promotion have been made in Brazil in recent decades, such as the National Basic Sanitation Law (2007) and the National Solid Waste Policy (2010) [18]. However, much work still needs to be done, especially considering Porto Alegre city. In contrast to diseases that do not have vaccines or treatments available, environmental interventions to reduce disease burden linked to inadequate WASH already exist [14] and can be immediately implemented in the presence of political will, intersectoral and public-private partnerships, and resource allocation [14]. Health sector and scientists must advocate the critical, and still underused, health-cost saving potential of sanitation [17]. Finally, we stress that human, animal, and environmental factors must be addressed together in health promotion policies focused on controlling parasitic infections [26].

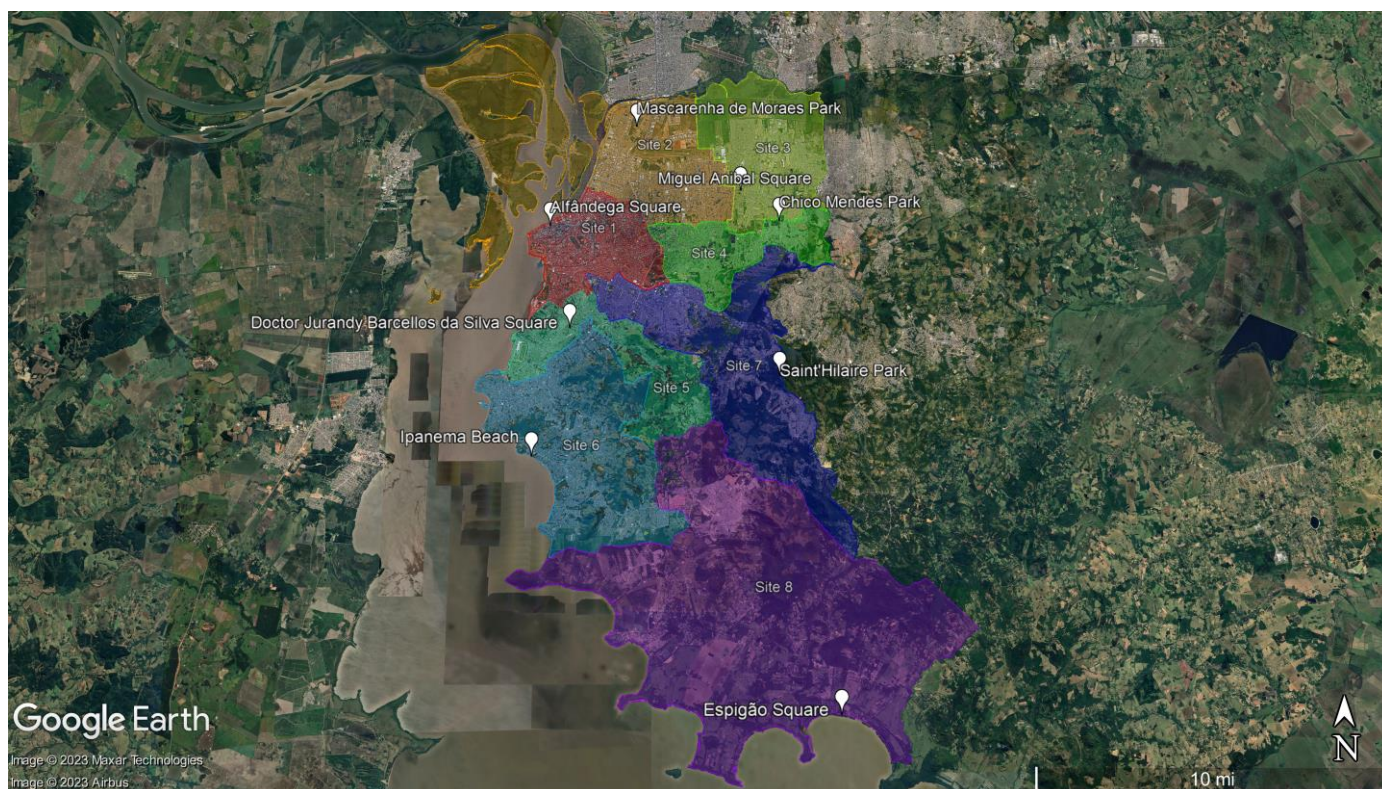
## 4. Materials and Methods

### 4.1. Legal Aspects

Soil samplings were authorized by the Secretaria do Meio Ambiente e Infraestrutura of Porto Alegre city and by the Sistema de Autorização e Informação em Biodiversidade—SISBIO (Instituto Chico Mendes de Conservação da Biodiversidade—ICMBio, Ministério do Meio Ambiente, Brazil): SISBIO No. 82718-1. The samplings performed at the Parque Natural Municipal Saint'Hilarie was also authorized by the institutional leaders. The animal taxa sampled in this study were registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen (Ministério do Meio Ambiente, Brazil): registration code A6C2812.

### 4.2. Study Area and Soil Sampling

Porto Alegre, located in the extreme south of Brazil, has an estimated population of 1,492,530 people, and includes areas covered by the Pampa and Atlantic Forest biomes [50]. With the objective of performing soil samplings at sites from various landscapes in Porto Alegre's territory, we based our sampling on the division of the eight planning regions of Porto Alegre city [51], as shown in Figure 1. For each of the eight regions, a park or public square with intense circulation of humans and animals was chosen for the soil samples. Specifically, the study sites were: Alfândega Square ("Praça da Alfândega"; site 1), Mascarenhas de Moraes Park ("Parque Mascarenhas de Moraes"; site 2), Miguel Anibal Genta Square ("Praça Miguel Anibal Genta"; site 3), Chico Mendes Park ("Parque Chico Mendes"; site 4), Doctor Jurandir Barcellos da Silva Square ("Praça Doutor Jurandir Barcellos da Silva"; site 5), Ipanema Beach ("Praia de Ipanema", a freshwater beach; site 6), Saint'Hilarie Park ("Parque Natural Municipal Saint'Hilarie"; site 7), and Espigão Square ("Praça do Espigão"; site 8). Table 1 details basic information on each sampling site.



**Figure 1.** Study area showing the eight planning regions of Porto Alegre city. Each region is highlighted in a different color. Sampling sites (detailed in Table 1) are indicated by white markers.

Samples were collected twice at each site: once in the winter of 2022 (July to September) and once in the summer of 2023 (January to March). For each season and from each study site, ten points were selected for soil sampling, considering places with indicatives of human and animal circulation. At each point, 150 g of topsoil was collected according to our pilot study (see Ziliotto et al. [52] for detailed methods). The samples were placed in sterile plastic tubes, and packed in a Styrofoam box, and then transported to the laboratory for microscopic and molecular analyses. Eighty samples were collected in winter and 80 in summer (ten samples from each sampling site per season), for a total of 160 soil samples.

Finally, to record data on biotic and abiotic factors that influence the presence/absence of soil nematodes, STH, and other parasites, we applied a previously described form to assess of socio-environmental and anthropic activity at each sampling site [52].

#### 4.3. Microscopy Analysis of Soil Samples

We recognize that there are a variety of effective techniques for detecting parasites [46–49,53]. In this study, microscopic analysis was used for the detection of STH larvae and eggs as it is considered the gold standard technique for STH studies [54]. In brief, soil samples were subjected to spontaneous sedimentation, stained with 2% Lugol, and analyzed under a microscope at 100× and 400× magnification, according to the Rugai's method [55] adapted for soil samples [31]. These analyses were performed by two trained microscopists. The methodological details used in this study were described by Ziliotto et al. [52]. The references used for the identification of the parasites were Neves [56], De Carli [57], Mariano et al. [58], and CDC [59]. As mentioned above, a total of 160 soil samples were analyzed. The samples were analyzed in duplicate (two slides for each sample, one slide for each microscopist), totaling 320 slides analyzed during the study.

#### 4.4. DNA Extraction and Molecular Analysis

For each sampling site, 50 g of topsoil from each of the ten sampling points was pooled. A small fraction of this pooled sample was subjected to DNA extraction using the Invitrogen™ PureLink™ Microbiome DNA Purification Kit for soil samples, following the manufacturer's recommendations. DNA extraction was performed in quadruplicate for each of the eight sampling sites (with each replicate corresponding to the pooled ten samples of the respective site, as previously mentioned), This resulted in 32 DNA samples from winter samplings and 32 DNA samples from summer samplings, for a total of 64 DNA samples.

Using the resulting DNA samples, we performed molecular analysis (polymerase chain reaction—PCR) to verify the presence of *Toxoplasma gondii* DNA in the samples. Conventional PCR targeting a non-coding fragment of 529 base pairs repeated in *T. gondii* genome was carried out according to the protocol of the Brazilian Ministry of Health for the investigation of *T. gondii* in environmental and food samples [60]. The primers used were: Tox4 5'-GCTGCAGGGAGGAAGACGAAAGTTG-3' and Tox5 5'-CGCTGCAGACACAGTGCATCTGGATT-3' [61]. According to the protocol, this technique showed 89% sensitivity and 91% of specificity. The PCR products were analyzed on a 1.5% agarose gel under UV light. The presence of a 529 base-pair band represents a positive result for *T. gondii* DNA [60].

#### 4.5. Statistical Analysis

The proportions of samples positive for non-pathogenic nematode larvae and pathogenic parasite structures between summer and winter were compared using Fisher's exact test. Tests were performed using WinPepi version 11.6 [62], and a *p*-value  $\leq 0.05$  was considered statistically significant.

### 5. Conclusions

This study described updated data on the presence/absence of various pathogenic parasites and non-pathogenic nematodes in soil samples from parks and public squares in Porto Alegre city, Brazil. These results highlight the importance of monitoring the prevalence of soil-transmitted parasites in places subjected to high human and animal circulation, which increases the risk of infection. Improvements in socio-environmental conditions, especially in the sanitation infrastructure of Porto Alegre are mandatory. Finally, our findings also suggest that climate change may affect soil biodiversity, favoring some groups of pathogenic parasites (e.g., STH), that may be more resilient to environmental changes and favored by higher temperatures.

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**CAPÍTULO VI**  
*Considerações Finais*

## DISCUSSÃO

A prevalência e a transmissão de doenças tropicais negligenciadas (DTN) se relacionam diretamente com a violação de direitos humanos como acesso à água potável, moradia e educação. Além de garantir proteção física e espaço apropriado, uma moradia adequada deve promover a saúde, garantindo acesso a água e tratamento de esgoto, formas de armazenar comida, energia e proteção contra riscos ambientais e vetores de doenças (Chastonay e Chastonay, 2022; United Nations, 2009). Além disso, doenças endêmicas podem prevalecer em ambientes que estejam sofrendo diferentes mudanças ambientais e climáticas, com alterações no equilíbrio ecológico (Ellwanger et al., 2020).

As DTNs ainda são bastante presentes no Brasil e suas consequências se estendem a diversos grupos populacionais vulneráveis. Um exemplo recente da gravidade das DTNs no Brasil recebeu bastante atenção da mídia Brasileira e exigiu diversas ações por parte do Governo Federal. O Ministério da Saúde decretou, em 2023, emergência de saúde pública em uma população de indígenas Yanomami em decorrência da grande quantidade de casos de desnutrição grave, aumento de casos de malária e infecção respiratória aguda, entre outros agravos (Ministério da Saúde, 2023). O problema se intensificou, segundo carta publicada pela associação Hutukara Yanomami ainda em 2022, devido ao avanço do garimpo ilegal na região da Amazônia, local da maior área territorial indígena do Brasil, com mais de 9.419.108 hectares de floresta tropical entre os Estados do Amazonas e Roraima (G1, 2023). A Amazônia possui uma alta diversidade de minerais e sua exploração ilegal resulta em desmatamento extensivo e poluição de água e solo, gerando intoxicação por mercúrio, doenças respiratórias, diarreia e maior exposição a diversos patógenos (Ellwanger et al., 2020). Na carta, a associação já denunciava a piora do quadro sanitário na Terra Indígena e alertava para o aumento de morte infantil por malária e aumento de registro de verminoses causado pela falta do medicamento Albendazol, um anti-helmíntico muito utilizado para o tratamento da infecção por diferentes verminoses, nos postos de saúde da região (Instituto Socioambiental, 2022). Também era denunciado o alarmante indicador de apenas 10% das comunidades com acesso adequado à água potável (G1, 2023). A falta de medicamento para tratamento adequado fez com que a população indígena atingisse níveis extremos de infecção por *A.*



*lumbricoides*. A desassistência sanitária dessa população durante os últimos anos resultou em mortes por desnutrição, diarreia e doenças tratáveis que já haviam sido controladas anteriormente, quando havia destinação adequada de recursos à saúde (G1, 2023). Apesar dos pontos mencionados acima terem sido observados na região Amazônica, geograficamente muito distante do Sul do Brasil, onde o presente trabalho foi realizado, eles exemplificam bem a gravidade da situação sanitária vivenciada atualmente por várias parcelas da população Brasileira, conforme apresentado no Capítulo I desta dissertação. Além disso, como amplamente discutido no Capítulo III, há uma forte conexão entre infecções parasitárias e problemas nutricionais, algo que ficou evidente entre a população Yanomami neste início do ano de 2023.

O Sistema Único de Saúde (SUS) visa promover o acesso universal à saúde, com prestação descentralizada de serviços de saúde e gestão federal, estadual e municipal, sendo considerado, por esta razão, como uma importante política de promoção de inclusão social (Brasil, 2014). Considerando a complexidade da incidência, prevenção e tratamento de doenças endêmicas no Brasil, o atendimento das Unidades Básicas de Saúde do SUS é extremamente importante, pois deve estar em consenso com as diferentes necessidades apresentadas em cada comunidade. A partir da implementação do plano integrado de ações estratégicas de eliminação da Hanseníase, Filariose, esquistossomose e oncocercose como problemas de saúde pública, tracoma (infecção bacteriana ocular) como causa de cegueira e controle das geo-helmintíases, lançado em 2012, foi adotada a estratégia de quimioprofilaxia preventiva para as geo-helmintíases, com o objetivo de reduzir a carga dessas doenças por meio de tratamento coletivo de escolares que vivem em localidades com condições precárias de saneamento ambiental, além do tratamento com anti-helmínticos (Brasil, 2021). Entre 2013 e 2018, o Plano Brasil sem Miséria estabeleceu ações de saúde voltadas para crianças de 5 a 14 anos de escolas públicas para promover a superação de iniquidades que incluíam o controle de doenças relacionadas e perpetuadoras da pobreza, nas quais se incluíam tuberculose, hanseníase, esquistossomose, helmintíases e tracoma, buscando acesso à prevenção, diagnóstico e tratamento nos municípios endêmicos (Brasil, 2014; Brasil, 2021).

O êxito de políticas públicas e de programas para a prevenção (vacinas e controles de vetores) e tratamento (antibióticos e medicação profilática) de doenças endêmicas infecciosas fez com que ocorresse forte redução nos índices de mortalidade da população relacionados ao que se consideram doenças características dos países subdesenvolvidos (Brasil, 2014). Porém, no Brasil, o quadro de erradicação é bastante complexo e ainda se observam prevalências preocupantes dessas doenças (Barreto, 2011; Brasil, 2014). O Brasil não apresenta dados oficiais atualizados sobre a prevalência de geo-helminthiases na população, sendo o último dado oficial divulgado o Inquérito Nacional de Prevalência da Esquistossomose mansoni e Geo-helminthoses (Katz, 2018). Conforme detalhado no Capítulo II, no Rio Grande do Sul a adesão ao Inquérito foi baixa. Apenas 36,9% do total previsto de amostras foram incluídas. Os dados avaliados demonstraram taxas de amostras positivas de 0,01% para ancilostomídeos, 0,56% para *A. lumbricoides* e 0,68% para *T. trichiura*. Apesar de os dados indicarem uma baixa prevalência de geo-helminthiases no estado, não podemos concluir com segurança que esses dados representem a situação epidemiológica durante o período do estudo, considerando a baixa adesão e o pequeno número de municípios incluídos ( $n= 14$ ). Dados obtidos através de uma revisão integrativa de trabalhos realizados entre 2010-2020 sobre prevalência de geo-helminthiases e condições socioambientais associadas no Brasil indicam uma prevalência de infecção em 19% da população, sendo *A. lumbricoides* o parasita mais prevalente (68%). Os principais fatores relacionados às infecções foram saneamento inadequado, hábitos de higiene insuficientes e baixa renda (Moreira et al., 2021).

Considerando as diversas intersecções geradas pelas causas e consequências das DTNs e outras doenças endêmicas no Brasil, as medidas para prevenção devem ser intersetoriais e o controle e vigilância devem ser priorizados (Meurer e Coimbra, 2022). As análises de geo-helminthos presentes no solo são importantes para a avaliação de todas as possíveis fontes de contaminação e podem apresentar informações sobre as condições sanitárias do local de análise, que auxiliam na prevenção de possíveis surtos (Tchakounté et al., 2018).

A técnica de Rugai adaptada para solos (Carvalho et al., 2005) possui baixo custo e se demonstrou bastante efetiva para a recuperação de ovos de *A. lumbricoides*, ancilostomídeos e *T. trichiura*, além de larvas de ancilostomídeos e *Strongyloides stercoralis*, conforme

detalhado nos trabalhos apresentados nos Capítulos IV e V. A técnica de microscopia para detecção de ovos e larvas ainda é considerada o padrão ouro e possibilita uma análise fácil e com baixos custos, porém apresenta uma baixa sensibilidade (Holmstrom et al., 2017; Khurana et al., 2021). Essas técnicas têm sido adaptadas para se tornarem cada vez mais rápidas e fáceis de aplicação, através de formatos digitais que apresentam alta sensibilidade (Khurana et al., 2021). A identificação por anticorpos é limitada apenas para a identificação de hiperinfecção por *S. stercoralis*. Apesar de técnicas como microscopia de imunofluorescência indireta (IFAT) e teste de aglutinação de partículas de gelatina (GPAT) apresentarem alta sensibilidade (81 – 98%), é necessário que larvas vivas e infectantes estejam presente em alta quantidade (Khurana et al., 2021).

As análises moleculares têm possibilitado grandes avanços nas técnicas de análises de parasitas. Diversos testes moleculares para detecção de parasitas foram desenvolvidos na última década, com um aumento de especificidade e sensibilidade gradual (O’Connell e Nutman, 2016). A análise molecular baseada em PCR pode ser útil na diferenciação de espécies de parasitas, no monitoramento de tratamento e também em estudos epidemiológicos que envolvam aspectos de diversidade genética, distribuição geográfica e susceptibilidade do hospedeiro, aumentando o conhecimento biológico, de distribuição e fatores de risco da doença (Tavares et al., 2011). Porém, além da técnica ainda apresentar custos mais elevados, é difícil estimar a qualidade da *performance* dos testes. Além disso, sua eficiência depende da preservação adequada da amostra e da qualidade de extração do DNA, que pode ser prejudicada pela diversidade de inibidores presentes tanto nas fezes quanto no solo (O’Connell e Nutman, 2016; Khurana et al., 2021; Gossen et al., 2019).

A aplicação do formulário para avaliação socioambiental e de atividade antrópica (detalhado no Capítulo IV) foi efetiva para ajudar na discussão das possíveis causas de contaminação nos diferentes locais de Porto Alegre em que ocorreram as coletas. As DTNs persistem por um conjunto de fatores biológicos e socioeconômicos que permitem que os vetores e patógenos prevaleçam perante mudanças no comportamento (animal e humano) e no ambiente físico (Manderson et al., 2009). Portanto, conforme amplamente discutido ao longo desta dissertação, é necessário que as abordagens de estudo, combate e prevenção dessas

doenças sejam feitas considerando a perspectiva de Saúde Única (*One Health*), abrangendo o conjunto de fatores humanos, animais e ambientais que influenciam a incidência e a disseminação das DTNs em cada ambiente (Ziliotto et al., 2022).

## CONCLUSÃO

Considerando todos os fatores abordados nessa dissertação, fica evidente a complexidade das questões referentes às DTNs. A prevalência de infecção por geo-helmintos ainda é bastante alta no Brasil, embora tenha apresentado uma ligeira queda nos últimos cinco anos (Moreira et al., 2021). É interessante que essa discussão seja feita considerando os mais diferentes cenários brasileiros, além de incluir os fatores que contribuem para as diferentes incidências apresentadas no país.

As DTN e doenças parasitárias são influenciadas pelos processos ligados à urbanização e mudanças no ambiente antropogênico (Manderson et al., 2009; Almeida et al., 2020). Porto Alegre apresenta uma série de questões complexas e diversas referentes a desigualdades de acesso à moradia e ao bem-estar urbano, assim como é visto em outros centros urbanos. Dessa forma, é interessante estratificar a cidade e analisar as disparidades socioeconômicas e ambientais entre as regiões (Salata et al., 2022; Lahorgue et al., 2022).

O controle de vetores de doenças em áreas urbanas e de habitação inadequada não pode ser alcançado apenas através de políticas de saúde. Tais esforços devem ser totalmente integrados em políticas amplas que incorporem a mobilização de sociedade, saúde e educação ambiental, melhorias em habitações e esgotos. A vigilância deve ser priorizada e intensificada, considerando os grupos populacionais vulneráveis e áreas geográficas que apresentam maior morbidade, incapacidade e mortes prematuras por DTNs (Barreto et al., 2011; Meurer e Coimbra, 2022).

É necessário que pesquisas e investimentos sejam esforços contínuos para a implementação de intervenções e programas de controle que garantam evidências para informar a adoção efetiva, sustentada e incorporada de intervenções eficazes nas comunidades (Manderson et al., 2009). Para além do controle de doenças, o método mais eficaz de diminuir a prevalência de geo-helmintos com eficiência é o acesso universal ao saneamento ambiental e moradia adequada (Chastonay e Chastonay, 2022). No âmbito municipal, algumas medidas podem ser tomadas pensando na melhora da qualidade de água, como atualização do Plano Municipal de Saneamento Básico considerando áreas de interesse social e ambiental e

implementação e manutenção de monitoramento em diferentes regiões da cidade (Zaneti et al., 2022). Considerando os pontos mencionados acima e os principais achados dos trabalhos que compõem essa dissertação, conclui-se que:

I) A prevalência de parasitas e outros patógenos é influenciada por diversas questões socioambientais e urbanísticas nas cidades; dessa forma, é interessante pensar em soluções de prevenção e contingenciamento sob a perspectiva de saúde única (“One Health”).

II) O Rio Grande do Sul é um estado que apresenta diferenças econômicas, culturais, ambientais e urbanas que podem influenciar as diferentes prevalências de geo-helmintíases de cada município, por isso, são necessários dados robustos de contaminação ambiental, animal e humana no estado para pensar em estratégias de prevenção e os possíveis impactos da distribuição de geo-helmintos nesses diferentes contextos.

III) A infecção por geo-helmintos possui diversas consequências para o hospedeiro e pode causar a deficiência de ferro. A deficiência de ferro, por sua vez, pode atuar como um fator de risco para a infecção por geo-helmintos por prejudicar o sistema imune, aumentando a suscetibilidade a infecções, e pela manifestação do comportamento de geofagia, favorecendo a contaminação de parasitas no solo.

IV) Os geo-helmintos fazem parte do ecossistema e estão presentes em diversos pontos do ambiente, incluindo o Campus do Vale, local de coleta do estudo piloto desta dissertação. O desequilíbrio de fatores ambientais pode favorecer a prevalência e o desenvolvimento desses parasitas, afetando a população que circula no local. Por isso, a análise de fatores socioambientais durante a coleta pode ajudar o reconhecimento das diferentes incidências.

V) Porto Alegre apresenta uma alta incidência de ovos e larvas de geo-helmintos em locais públicos de alta circulação na cidade. É importante considerar os diversos fatores que contribuem para essa incidência, como problemas de moradia e falta de saneamento ambiental adequado.

## **PERSPECTIVAS**

- Realizar novas coletas nas oito regiões de Porto Alegre durante o período do verão, comparando a prevalência dos parasitas analisados entre inverno e verão.
- Finalização do artigo “Soil-transmitted parasites in soil samples from Porto Alegre city, southern Brazil” e envio para publicação na revista *One Health*.
- Avaliar diferentes genes do sistema imune considerando a susceptibilidade de infecção por parasitas na população de Porto Alegre.
- Explorar as relações entre variabilidade genética e diferentes desfechos de infecção parasitária.

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## **ANEXO A – produção complementar**



## BIOMEDICAL SCIENCES

# Synthesizing the connections between environmental disturbances and zoonotic spillover

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**Abstract:** Zoonotic spillover is a phenomenon characterized by the transfer of pathogens between different animal species. Most human emerging infectious diseases originate from non-human animals, and human-related environmental disturbances are the driving forces of the emergence of new human pathogens. Synthesizing the sequence of basic events involved in the emergence of new human pathogens is important for guiding the understanding, identification, and description of key aspects of human activities that can be changed to prevent new outbreaks, epidemics, and pandemics. This review synthesizes the connections between environmental disturbances and increased risk of spillover events based on the One Health perspective. Anthropogenic disturbances in the environment (e.g., deforestation, habitat fragmentation, biodiversity loss, wildlife exploitation) lead to changes in ecological niches, reduction of the dilution effect, increased contact between humans and other animals, changes in the incidence and load of pathogens in animal populations, and alterations in the abiotic factors of landscapes. These phenomena can increase the risk of spillover events and, potentially, facilitate new infectious disease outbreaks. Using Brazil as a study model, this review brings a discussion concerning anthropogenic activities in the Amazon region and their potential impacts on spillover risk and spread of emerging diseases in this region.

**Key words:** Amazon Forest, biodiversity, disease ecology, outbreak, spillover, zoonosis.

## INTRODUCTION

A robust set of evidence shows that conservation of biodiversity and of balance in ecosystems and food webs reduces the risk of emergence and spread of infectious diseases of zoonotic origin, in addition to contributing to human well-being in general (Ostfeld 2009, Keesing et al. 2010, Pecl et al. 2017, IPBES 2020). From a practical point of view, limiting anthropogenic activity in environments with high abundance

and diversity of species contributes to the maintenance of human and environmental health, containing emerging infectious diseases by multiple ecological mechanisms. On the other hand, anthropogenic disturbances (e.g., deforestation, habitat fragmentation, intensive agricultural practices, unplanned urbanization) indeed affect this balance, facilitating the emergence of new pathogens and the spread of diseases (Ellwanger et al. 2020, IPBES 2020). On a global scale, land-use change was the

major driver of more than 30% of new emerging infectious-disease events since 1960 (IPBES 2020).

Currently, there are more than 7.7 billion humans on Earth. Projections indicate that the world population will reach 9.4 to 10.2 billion people by 2050 (Boretti & Rosa 2019). Economic, political, social, and cultural factors dictate human activities in the natural environment, many of which cause damage and disturbances to the environment and to animal populations. The food, water, and consumption demands of the global population are putting great pressure on nature and triggering a range of environmental problems, with expected exacerbation of this scenario in the near future due to the growing world population (Conijn et al. 2018, Boretti & Rosa 2019, Pastor et al. 2019).

The connections between environmental disturbances and infectious diseases are increasingly worrying because efforts to contain deforestation, climate change, and other environmental impacts are still very modest. Globally, habitat loss and extinction rates are on the rise (Ceballos et al. 2015, Newbold et al. 2016, Powers & Jetz 2019), and the detrimental effects of climate change on the human population and other species are increasingly evident (Beyer et al. 2021, Ma & Yuan 2021). Concomitant with this scenario of environmental neglect, the number of emerging infectious disease events per decade is increasing (Jones et al. 2008). Table I shows several examples of infectious disease outbreaks associated with anthropogenic pressures on the environment and animal populations. Also, the economic impacts and losses of human lives related to Coronavirus Disease 19 (COVID-19), which is a zoonotic disease, make clear the magnitude and severity of the situation and the need to understand how to reduce the risks of new pandemics (Dobson et al. 2020, Holmes et al. 2021). As of February 2, 2022, COVID-19 had

caused more than 381 million infection cases and 5,688,009 deaths worldwide (Dong et al. 2020, Johns Hopkins University 2022). It is increasingly clear that when a pathogen emerges in a given human population after an event of zoonotic spillover, even in a remote location, the pathogen can quickly spread globally by international air travel and other transport systems, especially in situations of high connectivity between remote regions and large urban centers.

Zoonotic spillover is a phenomenon characterized by the transfer of pathogens between different species (usually non-human animals to humans), which may result in new infectious diseases if biological and demographic conditions are conducive to the adaptation of the pathogen in the new species population. Spillover events are among the initial steps towards the emergence of new human infectious diseases, outbreaks, and epidemics (Plowright et al. 2017, Ellwanger et al. 2019). Most of the pathogens (~60%) that affect humans are derived from microbial strains that previously circulated only in non-human animals (Jones et al. 2008), such as HIV (Keele et al. 2006), influenza A viruses (Krammer et al. 2018), Zika virus (Wikan & Smith 2016), Ebola virus (Leroy et al. 2005, Saéz et al. 2015), rubella virus (Bennett et al. 2020), *Echinococcus multilocularis*, *Trypanosoma cruzi* (Thompson 2013), hepatitis B virus (Rasche et al. 2016), MERS-CoV, SARS-CoV (Cui et al. 2019) and SARS-CoV-2 (Andersen et al. 2020), among many others (Montgomery & Macdonald 2020). Considering adenoviruses, phylogenetic analyses indicate that at least 16 B-type human adenoviruses (HAdV) had their original reservoir in great apes, some of them causing severe human disease. Also, it was proposed that the HAdV-B76 strain, which is associated with a human fatality in 1965, arose from recombination of a virus that infected humans, chimpanzees, and bonobos

**Table I. Examples of connections among human-related environmental disturbance, animal populations and infectious diseases.**

Anthropogenic pressures on the environment and animal populations*	Effect or association with disease emergence or dissemination	References
Habitat loss, deforestation, industrial agriculture, monoculture practices, mining, and other types of land-use changes	Forest fragmentation, fires and other disruptions of natural habitats of bats resulted in outbreaks of Nipah and Hendra viruses in Australasia	Field et al. (2001), Chua (2003), Epstein et al. (2006)
	Deforestation and road expansion were associated with increased human-biting rate of <i>Anopheles darlingi</i> (primary malaria vector) in the Peruvian Amazon	Vittor et al. (2006)
	Habitat fragmentation and biodiversity loss were associated with a higher prevalence of <i>Trypanosoma cruzi</i> infection among small mammals in an Atlantic Rain Forest landscape of Brazil	Vaz et al. (2007)
	Increasing of land cultivated for sugarcane and high annual mean temperature were associated with hantavirus pulmonary syndrome incidence in the Neotropics	Prist et al. (2016)
	Forest loss triggered increased risk of Kyasanur Forest disease (tick-borne viral hemorrhagic fever) in India	Walsh et al. (2019a)
	Habitat changes of putative wild rodent reservoirs and agriculture-related activities were associated with fatalities from Sabiã virus infection (two in 1990 decade and one in 2020), São Paulo State, Brazil	Ellwanger & Chies (2017), Malta et al. (2020)
	Land-use (e.g., habitat degradation) was associated with changes in parasite richness and prevalence, as well as co-infection patterns, of avian parasites	Reis et al. (2021)
	Agricultural and irrigation practices were associated with mosquito proliferation, with increases in Japanese encephalitis cases	Keiser et al. (2005)
	Deforestation for agriculture and cattle pasture was associated with development and dissemination of antibiotic resistance in the Amazonian soil microbiome	Lemos et al. (2021)
	Anthropogenic deforestation associated with the shortage of fruiting due to drought-triggered movement of fruit bats to livestock areas, infecting pigs and then humans with Nipah virus in Malaysia	Chua et al. (2002), Looi & Chua (2007)
	Colonial practices in Indigenous areas had a major impact on the health of Indigenous populations, who were exposed to various infectious diseases transmitted by European colonizers and explorers in American and African continents, for example	Valeggia & Snodgrass (2015), Owers et al. (2017)
	Mining, logging, illegal land grabbing and other types of land-use changes in Indigenous lands favors the transmission of SARS-CoV-2, malaria, sexually transmitted infections, and other infectious diseases in Amazonian Indigenous populations	Ellwanger et al. (2020), Vittor et al. (2021)
	In Brazil, political changes permissible to illegal activities (e.g., logging, mining, fires, weakening of Indigenous leaders) on Indigenous Lands ( <i>Terras Indigenas</i> ) exposed Indigenous and traditional communities to multiple infectious diseases, including COVID-19	Branca et al. (2020), Ferrante et al. (2020)
	Mining and other types of land-use changes were associated with Buruli ulcer (caused by <i>Mycobacterium ulcerans</i> infection) in southwestern Ghana	Wu et al. (2015)
	Agricultural practices exacerbated the risks of many infectious and parasitic diseases (hookworm, malaria, scrub typhus, spotted fever group diseases, schistosomiasis, <i>Trichuris trichuria</i> infection) in Southeast Asia	Shah et al. (2019)
	Mining related practices favored emerging infectious disease events in Africa, including Ebola outbreaks, with mining-associated political interests exacerbating such outbreaks	Wallace et al. (2016), Guègan et al. (2020), Ostergard Jr (2021)
	Monoculture and other current food systems practices expose populations to various health issues, including infectious and parasitic diseases (in some cases derived from malnutrition) and multi-resistant microbes at a global scale	Pradyumna et al. (2019), Everard et al. (2020)
	Gold mine workers are highly exposed to hantavirus infection, malaria and leishmaniasis in South America	Rotureau et al. (2006), Terças-Trettel et al. (2019)
	Agricultural systems bring some bat species (e.g., <i>Desmodus rotundus</i> vampire bat) closer to humans and domestic animals, increasing the risk of bat-borne infections, including rabies outbreaks	Rosa et al. (2006), Kuzmin et al. (2011)
	Infectious diseases events were associated with changes in forest cover and oil palm expansion at a global scale	Morand & Lajaunie (2021)
	Sugar cane monoculture favors some opportunistic rodents, favoring hantavirus infection in humans	Figueiredo et al. (2010)
	Gold mining-associated activities and settlements favor the spread of infectious diseases (e.g., tuberculosis, HIV/AIDS and other sexually transmitted infections, rabies, vector-borne diseases) in Australia, Africa, North America, and South America	Ogola et al. (2002), Eisler (2003)
	Anthropogenic disturbances (e.g., crop plantation, removal of vegetation cover for cattle raising) lead to simplification of ecosystems (biodiversity loss) and thus favor populations of opportunistic/generalist animal species that can transmit hemorrhagic fever viruses to humans	Mills (2006)
Co-circulation of Araraquara and Juquitiba hantaviruses in rodents was detected in the Brazilian Cerrado biome, with agricultural practices increasing the risk of human hantavirus infection	Guterres et al. (2018)	

**Table I. Continuation.**

Climate change and extreme weather events	Climatic anomalies (with heavy rainfall and eventually flooding after periods of drought) and increase in air and sea surface temperatures triggered outbreaks of Rift Valley Fever in Africa	Anyamba et al. (2001), Martin et al. (2008)
	Fossil fuel-related climate change associated with air pollution favor the occurrence of respiratory infections (e.g. pneumonia, fungal infection, Hantavirus respiratory disease), especially in children	Mirsaeidi et al. (2016), Brugha & Grigg (2014)
	An increase in coccidioidomycosis cases in Arizona from 1998 to 2001 was associated with climatic and environmental changes such as wind, mean temperature, dust and rainfall because these factors affect the abundance of fungal arthrospores of <i>Coccidioides</i> species in the air	Park et al. (2005)
	Extreme weather events, in association with de-urbanization, were associated with higher risk of flood-related non-cholera diarrhea in lower hygiene and sanitation groups in a post-flood period in Dhaka, Bangladesh. Rotavirus, <i>Escherichia coli</i> , <i>Campylobacter</i> and <i>Aeromonas</i> were the most common pathogens causing non-cholera diarrhea episodes	Hashizume et al. (2008)
	Climate change was associated with increased human cases of Lyme disease	Germain et al. (2019)
	Fossil fuel-related climate change will change the distribution patterns of zoonotic and vector-borne diseases in the world in a way difficult to accurately predict, but in general favoring the spread of these diseases on a global scale	Wilkinson et al. (2007), Greer et al. (2008), Dantas-Torres (2015), Wilke et al. (2019b)
	Climate change and land-use change were associated with an increased risk of acute gastrointestinal diseases	Brubacher et al. (2020)
	Climate abnormalities and melting of permafrost released <i>Bacillus anthracis</i> , the etiological agent of the anthrax disease, infecting reindeer, cattle, and humans	Timofeev et al. (2019), Maksimović et al. (2017), Stella et al. (2020)
	Temperature rise alters the distribution, optimal conditions for breeding, growth and survival of <i>Schistosoma</i> -related snails, and such conditions were associated with increased risk of spread and transmission of schistosomiasis	Kalinda et al. (2017)
	Hunting, industrial livestock production, bushmeat practices, and other types of wildlife exploitation	Bushmeat-related practices triggered the SARS-CoV emergence and outbreak in Asia in 2003 and 2004
Pervasive contact with wildlife (e.g., hunting, bushmeat-related practices), in association with forest fragmentation and loss, triggered Ebola virus disease outbreaks in Africa		Judson et al. (2016), Olivero et al. (2017), Rulli et al. (2017)
Coccidiomycosis cases resulted from armadillo hunting		Costa et al. (2001), Brillhante et al. (2012), Capellão et al. (2015)
Poultry and livestock are sources of multiresistant <i>E. coli</i> isolates with clinical importance in China		Yassin et al. (2017)
Livestock and poultry are sources of antimicrobial resistance genes of <i>Enterococcus</i> spp. isolates in Lithuania		Ruzauskas et al. (2009)
An animal-based agriculture river system was associated with antimicrobial resistance of <i>Salmonella</i> sp. in Brazil, with multi-resistance found in 18% of isolates		Palhares et al. (2014)
Poultry and food products (e.g., retail meat, sushi, ready-to-eat foods) are sources of multi-resistant and methicillin-resistant <i>Staphylococcus aureus</i> isolates in Europe		Nemati et al. (2008), Li et al. (2019)
A swine production system was associated with anti-microbial resistance in <i>Campylobacter</i> spp., <i>E. coli</i> and <i>Enterococcus</i> spp. in Australia		Hart et al. (2004)
Antimicrobial resistance and virulence genes of <i>Streptococcus</i> and <i>Salmonella enterica</i> were detected in isolates obtained from dairy cows in Asian countries		Chuanchuen et al. (2010), Ding et al. (2016)
Animals raised for consumption (e.g., chickens, pigs, cattle) use the majority (73%) of antimicrobials sold in the world, and these animals are major sources of multiple multi-resistant microbes, especially in developing countries and emerging economies, such as India, China, Brazil and Iran		Van Boeckel et al. (2019)
Hunting, cleaning and eating of armadillos were associated with the development of Hansen's disease ( <i>Mycobacterium leprae</i> infection) in humans		Capellão et al. (2015), Van Vliet et al. (2017), da Silva et al. (2018)
Human-promoted elephant-livestock interface increased anthrax transmission risk in India		Walsh et al. (2019b)
The wildlife exploitation through hunting and trade of threatened wildlife species favors close contact between humans and wildlife, which are contributing factors of spillover events		Johnson et al. (2020)
Human interaction with animal species (wildlife exploitation, animal trade, livestock industry?) triggered the SARS-CoV-2 emergence and the related COVID-19 pandemic		Lam et al. (2020), Zhang & Holmes (2020), Zhang et al. (2020), Holmes et al. (2021)
Livestock/agro-pastoral activities were associated with occurrence of zoonotic diseases, such as brucellosis, Q-fever, and Rift Valley fever, affecting both humans and livestock in Ethiopia		Ibrahim et al. (2021)
Hunting, bushmeat and related activities caused the HIV spillover from wild primates to humans in Africa (around 1920 or before), later (around 1960) spreading around the world as a result of road expansion and globalization, among other social and economic factors		Hahn et al. (2000), Gray et al. (2009), Faria et al. (2014), Gryseels et al. (2020)
Reassortment of different influenza viruses in swine creates new subtypes of influenza, the causative agent of the Spanish flu (1918) and the swine flu (2009); Influenza reassortment events are facilitated by livestock practices	Tomley & Shirley (2009), Shi et al. (2014)	

**Table I. Continuation.**

Urbanization, de-urbanization, and environmental changes due to infrastructure expansion	The construction of the Binational Itaipu Reservoir contributed to the proliferation of <i>Anopheles</i> mosquitoes and the increase in <i>Plasmodium vivax</i> malaria cases in the region of the Paraná River (Brazil)	Falavigna-Guilherme et al. (2005), Leandro et al. (2021)
	In Fiji, the presence of <i>Leptospira</i> antibodies was associated with different environmental and socio-demographic variables such as living in villages, lack of access to treated water, working outdoors, living in rural areas, high poverty rates, contact with animals, among other factors	Lau et al. (2016)
	The construction of dams was associated with malaria transmission in sub-Saharan Africa	Lautze et al. (2007), Kibret et al. (2019)
	Poorly planned urbanization, presence of waste, and precarious sanitation conditions were linked to the proliferation of <i>Aedes aegypti</i> mosquitoes and circulation of urban arboviruses (e.g., dengue, chikungunya and zika)	Almeida et al. (2020)
	Poor housing conditions in association with loss of habitat and food sources favor the infestation of human dwellings by triatomine bugs, transmitters of <i>T. cruzi</i> (Chagas disease agent)	Starr et al. (1991), Schofield et al. (1999), Lima et al. (2012), Crocco et al. (2019)
	Human contact with wildlife that resulted from mining and entering caves promoted Marburg virus outbreaks in Africa (infection source linked to bats in caves and mines)	Bausch et al. (2003), Pawęska et al. (2018), Amman et al. (2020)
	Higher risk of schistosomiasis infection due to the construction of dams (water blockage) in Africa	Sokolow et al. (2017)
	Overcrowding, environmental contamination, exposure to disease vectors and lack of public health infrastructure favors the transmission of infectious and parasitic diseases in Indigenous populations in many countries	Gracey & King (2009)
	Marginalized and Indigenous peoples in the United States and Brazil experience disproportionate burdens of COVID-19 (both morbidity and mortality) due to social injustice, lack of vaccines and public health infrastructure, and political weakening of Indigenous leaders	Santos et al. (2020), Costa et al. (2021), Hiraldo et al. (2021)
	Infrastructure problems, water contamination and poverty favor infectious and parasitic diseases in Indigenous populations of the Arctic	Hotez (2010)

**\*Many types of anthropogenic pressures on the environment and on animal populations have been grouped by categories for better organization of the table. However, we stress that in many examples, different categories of anthropogenic actions are acting in association to favor the emergence or spread of disease. At the global level, intensive causes/practices of land-use changes (e.g., logging, mining, industrial livestock production, fossil fuel extraction, deforestation) certainly have a much greater impact as drivers of spillover and emerging infectious disease events than individual practices of consumption and behavior.**

(Hoppe et al. 2015, Dehghan et al. 2019, Kremer 2021).

In this article, the expression ‘zoonotic spillover’ will be used to refer to the introduction of a pathogen into the human population from a different animal species. However, it is essential to emphasize that spillover is a complex phenomenon. There are different pathways of spillover events. For example, a pathogen can be transmitted from one species (source host) to another (recipient host) directly, without an intermediate species. Alternatively, some spillover events involve an intermediate species (intermediary host) that acts as a ‘bridge’ for the transmission of the pathogen between the source host and the recipient host. The intermediary host can be a vertebrate species or an invertebrate animal (e.g., mosquito,

tick). More than one intermediate host may be involved in the spillover event. Also, the spillover can involve the environment. In this case, the recipient host is infected by the pathogen that has been released into the environment by the source or intermediate host (Borremans et al. 2019, Ellwanger & Chies 2021).

The association between anthropogenic activity and emerging infectious diseases has been increasingly recognized by the scientific community and by the general population since the beginning of the COVID-19 pandemic, with some positive impact on public concern and awareness about nature and environmental issues (Rousseau & Deschacht 2020, Severo et al. 2021). A search on the PubMed database using in association the terms “environmental change” and “pandemic” resulted in 1974

documents published in 2021, a huge increase compared to the 64 documents published in 2019 (<https://pubmed.ncbi.nlm.nih.gov/>; search performed on February 2, 2022). However, the connections and ecological mechanisms linking environmental disturbance and increased risk of zoonotic spillover events are not always explored in scientific publications. Synthesizing the sequence of basic events involved in the emergence of new human pathogens is important to guide the understanding, identification, and description of key aspects of human activities that can be changed to prevent new outbreaks, epidemics, and pandemics. This knowledge is critical for researchers from different fields. Thus, the main aim of this review is to synthesize the principal connections between environmental disturbances and increased risk of spillover events. In this article, 'environmental disturbance' refers to disturbance, damage or imbalance caused by human activity on natural landscapes, urban and rural areas, animal populations, or ecosystems.

Considering the multiple dimensions surrounding the association between anthropogenic activity and infectious diseases, this article was written with the collaboration of authors from multiple fields, a strategy aligned with the One Health perspective. In the first part of this article, we briefly discussed the relationship between biodiversity and spillover risk. Subsequently, the connections between environmental disturbances and spillover events are reviewed. Considering the authors' expertise on tropical ecosystems in the Brazilian context, this review also brings a discussion concerning anthropogenic activities in the Amazon region and their potential impacts on spillover risk and spread of emerging infectious diseases in this region. This article therefore differs from the literature on emerging diseases because it brings together basic information on

anthropogenic activities that facilitate zoonotic spillover events in different contexts and countries, and it provides an analysis focused on a specific highly biodiverse biome - the Amazon Forest.

## BIODIVERSITY AND SPILLOVER RISK

Biodiversity can be associated with an increased risk of infectious diseases in some situations. For example, this can occur by adding new infectious agents or carriers to the environment (biodiversity as a 'source' of pathogens), or through the incorporation of new (host) species into a given environment, or by increasing food sources for disease vectors and thus contributing to their proliferation (Keesing et al. 2006). A good discussion regarding the complex influences of biodiversity on infectious diseases can be found in Rohr et al. (2020). Although on some occasions biodiverse environments can be associated with increased risk of infectious diseases, biodiversity *per se* is not the cause of emerging infectious diseases. On the contrary, biodiversity usually confers protection to human health.

High-biodiversity ecosystems 'dilute' the density of reservoir hosts and competent vectors, minimizing the contact between reservoir hosts and vectors and reducing the prevalence or load of pathogens in these hosts and vectors, thus decreasing the risk of zoonotic infections. Greater richness and diversity of predators and competitors can also contribute to the control of species that are both adapted to human-modified environments and have the potential to transmit zoonotic pathogens (Schmidt & Ostfeld 2001, Keesing et al. 2006, Ostfeld 2009, Pongsiri et al. 2009, Civitello et al. 2015, Kilpatrick et al. 2017). The phenomenon in which high species diversity reduces the risk of



infectious diseases is called the 'dilution effect' (Keesing et al. 2006).

A good example of the dilution effect can be found in Lyme disease, which is caused by the *Borrelia burgdorferi* infection and is transmitted by ticks in the genus *Ixodes*. Different mammals are natural hosts of *B. burgdorferi* in nature, including the white-footed mouse (*Peromyscus leucopus*), a highly competent reservoir. The disease affects human populations living in the USA, Canada, and European nations, among other countries. In the presence of a great diversity of reservoir hosts in nature (white-footed mouse plus other hosts), ticks feed on the blood of different hosts with varied competence for *Borrelia* transmission (many with a low reservoir competence), thus 'diluting' the number of infected ticks and consequently the risk of Lyme disease (LoGiudice et al. 2003, Ozdenrol 2015, Keesing & Ostfeld 2021). In brief, high host diversity including poor competent hosts dilutes the infection risk exerted by the few highly competent hosts. On the other hand, the risk of disease increases as the diversity of *Borrelia* hosts declines and the density of competent reservoir hosts increases in a particular area (LoGiudice et al. 2003, Keesing & Ostfeld 2021).

The impact of host diversity on the disease risk was observed in other models beyond Lyme diseases. The risk of human infection by West Nile virus and Hantavirus decreases as the diversity of their hosts (wild birds and rodents, respectively) increases. The opposite correlation can also occur, with the risk of human infection increasing as host diversity decreases (Mills 2006, Allan et al. 2009, Ostfeld 2009). These two additional examples highlight the dilution effect, indicating how biodiversity can 'dilute' the risk of zoonotic spillover events, protecting human health.

The dilution effect is strongly related to the number and relative abundance of taxa, namely taxonomic diversity (Naeem et al. 2012), which is the kind of "biodiversity" discussed above. However, it is essential to consider that other forms of biodiversity exist, with varied impacts on disease risk. According to Naeem et al. (2012), biodiversity can be classified on the basis of several dimensions, including taxonomic, phylogenetic, genetic, functional, spatial or temporal, interaction, and landscape diversities. These other dimensions of biodiversity can also affect spillover events and the spread of pathogens. For example, genetic diversity has contributed to the emergence of new pathogens or variants, as observed in the SARS-CoV-2 and HIV pandemics, facilitating the transmission and spread of the viruses to different countries (Faria et al. 2014, Andersen et al. 2020). Also, interaction diversity (e.g., competition, predation, parasitism) has an important influence on the risk of emergence and spread of zoonotic diseases (Vourc'h et al. 2012) because it modulates the contact between species, host immunity, transmission of pathogens and food webs.

It is also fundamental to stress that the dilution effect does not apply to all types of zoonotic diseases, being more closely related to diseases borne by vectors such as arthropods and rodents, as indicated by the examples mentioned above. Furthermore, the dilution effect may be scale dependent. The protective role of biodiversity on disease risk observed at the local scale may not be observed when the effect of biodiversity is analyzed at the global scale. Also, the ecological history of each disease is different and, in some cases, is either weakly dependent on the degree or dimension of biodiversity or its effect is only indirect (Rohr et al. 2020). Some diseases of zoonotic origin, but that are currently highly specialized

on the human host (e.g., measles, tuberculosis, pneumonia), have a weak relation with measures of biodiversity (Rohr et al. 2020).

Other factors (e.g., demography, social issues) also increase the complexity of the relation between biodiversity and zoonotic risk. Globally, countries with high biodiversity are often precisely those where the burden of zoonotic diseases can be observed most intensely. For example, Brazil is classically affected by multiple types of zoonotic vector-borne diseases (Magalhaes et al. 2020), despite being one of the most biodiverse countries in the world. Inadequate sanitary conditions and precarious public health systems, which are frequently observed in tropical developing countries, can override the dilution effect associated with high biodiversity, facilitating the emergence and spread of diseases in these countries (Ellwanger et al. 2021). In brief, the connections between biodiversity and zoonotic spillover are multiple and complex. Although some generalizations are possible, such as the dilution effect and the connections that will be discussed later in this article, each pathogen and spillover event must be analyzed according to its natural history and the context of its occurrence.

## **ALIGNMENT OF CONDITIONS CONDUCTIVE TO ZOOONOTIC SPILLOVER**

### **Role of animal groups**

An increased risk of spillover events is usually associated with particular animal orders, including Chiroptera and Rodentia, which are composed of species with supposed high 'zoonotic potential'. Also, anthropogenic modifications in landscapes favoring human contact with rodents and bats are usually associated with increased risk of zoonotic infection. However, some criticism concerning

these aspects is needed. Some animal species can indeed host a high load or diversity of zoonotic pathogens due to intrinsic biological characteristics (e.g., immune system factors, genetic proximity to humans) or due to ecological characteristics, such as the sharing of a habitat with humans or livestock. However, a greater load and variety of pathogens in certain reservoir hosts can be circumstantial and is not necessarily an intrinsic characteristic of a specific animal group. Bats and rodents are often considered highly competent in transmitting pathogens to humans because they harbor a great diversity of zoonotic pathogens, reproduce quickly and often inhabit human-related environments. Chiroptera and Rodentia are the most numerous orders of placental animals, which can increase the opportunity for the emergence of potential zoonotic agents from these animals (Luis et al. 2013, Han et al. 2016). Canidae and Felidae are families in the order Carnivora that also pose risks to the human population in terms of zoonotic spillover because their members (e.g., dogs, foxes, cats) host different zoonotic pathogens and frequently circulate in human-dominated areas (Han et al. 2021).

Stray dogs and cats find favorable conditions to proliferate in areas where urbanization has taken place in a disorderly way, and these animals are transmitters of zoonotic diseases in urban centers, especially due to soil contamination with the eggs and oocysts of parasites (*Toxocara*, *Trichuris*, *Toxoplasma*, *Cystoisospora* and *Taenia* genera, among others) released into the environment through animal feces. This is a particularly important problem for children because they come into greater contact with the soil in public squares and parks; this affects populations in China, South America, highly developed European nations, and elsewhere (Szwabe & Błaszowska 2017, Montoya et al. 2018, Fu et al. 2019, Saldanha-Elias

et al. 2019). Moreover, leishmaniasis is a major zoonotic disease in several Latin American countries. This disease is caused by *Leishmania* parasites, which have dogs as common reservoirs. The disease is transmitted by phlebotomine sandflies that proliferate in areas with a lack of environmental sanitation and an abundance of domestic animals, thus affecting people living in urban and peri-urban areas (Teodoro et al. 1999, Marcondes & Day 2019). These cases exemplify the role of the order Carnivora as an additional source of zoonotic pathogens.

Mammals in the order Rodentia were initially classified as the animal group with the highest number of zoonotic hosts, with ~11% of species having zoonotic potential. Highly competent rodent reservoirs show a fast life history profile, reaching sexual maturity and producing offspring at higher rates earlier in life as compared to non-reservoir rodents. Also, highly competent rodent reservoirs usually thrive in areas with high human population densities (Han et al. 2015, 2016). Similar to rodents, bats are usually considered to be of special zoonotic concern because they have high longevity, the colonies are numerous, and the share of viruses between different bat species is increased due to sympatry (Luis et al. 2013). Bats have immune systems with unique adaptations that allow these animals to harbor many viruses without themselves becoming sick, which contributes to making these animals of special concern regarding zoonotic risk (Hayman 2019, Subudhi et al. 2019).

However, it is necessary to consider some points regarding the role of the orders Rodentia and Chiroptera (especially bats) as disproportionate zoonotic reservoirs. Limited inflammatory responses, high population densities and gregarious social behaviors observed in some bat species may indeed facilitate pathogen transmission among bats,

especially viruses, contributing to the zoonotic potential of this group (Brook & Dobson 2015, Streicker & Gilbert 2020). On the other hand, the lack of knowledge about the immunity of other animal groups, including their ability to harbor pathogens asymptotically, may currently be biasing the conclusion that bats or rodents are especially competent in harboring and transmitting zoonotic pathogens. There is also high immunological variation among bat species, making generalizations about the ability of bats to transmit zoonosis a complicated task. Beyond bats and rodents, other animal groups can be of great importance for the transmission of zoonotic pathogens to humans, although they have been less considered and sampled in studies involving zoonotic diseases (Streicker & Gilbert 2020). A recent study by Mollentze & Streicker (2020) reported that the viral zoonotic risk was homogenous among mammalian and avian species when reservoir hosts of 415 RNA and DNA viruses were considered, this being the largest dataset to date. Bats and rodents were considered unexceptional zoonotic hosts, with the proportion of zoonotic viruses varying minimally across the taxonomic orders of the reservoirs that were analyzed (Mollentze & Streicker 2020).

Still concerning bats, human activity has effects beyond those expected from human-triggered changes in the sizes and population structures of these animals. Bats are highly sensitive to anthropogenic activity, which generates physiological stress in these animals. These physiological changes impact infection severity and pathogen shedding in bats, affecting their associated viral populations and risk of spillover events. For example, Plowright et al. (2008) observed that reproduction and nutritional stress in little red flying foxes (*Pteropus scapulatus*) increases the risk of Hendra virus infection in

these animals, potentially increasing the risk of human infection when these conditions occur. Pregnant and lactating female bats showed higher Hendra virus infection rates, and animals under nutritional stress showed higher infection prevalence, a result potentially derived from factors such as poor immune defense or greater contact with other animals while sharing food (Plowright et al. 2008). Furthermore, recent data have shown that the ecological conditions of the flying fox hosts of Hendra virus influence the timing, magnitude, and cumulative intensity of virus shedding, thus affecting the spillover risk (Becker et al. 2021). Based on these findings, changes in bat immunity derived from human-associated environmental disturbances (e.g., habitat loss, food shortages) can be considered a mechanism by which human activity can increase the risk of spillover events and zoonotic diseases, since these immunological changes can increase infection severity, viral shedding and infection rate in reservoir host populations. Moreover, this information indicates that the zoonotic risk attributed to a given host is *circumstantial* and not necessarily *intrinsic* to a specific animal group, with human-related interference on these hosts influencing the circumstantial zoonotic risk.

### **Host-associated factors, pathogen characteristics and the environmental context**

Several host-associated factors can increase or reduce the risks of spillover events, including pathogen load in the source or intermediate host, immunity or nutritional status of recipient host, similarity of pathogen receptors in the different hosts, and genetic/evolutionary distance between species. Spillover risk is also modulated by ecological conditions (e.g., habitat sharing by different species, changing patterns disease in reservoir populations, changing reservoir species behavior) and environmental

factors (e.g., landscape characteristics, environmental sanitary conditions, abiotic factors: temperature, humidity, rainfall). Adding more complexity to spillover risk, characteristics of the pathogens (e.g., virulence, transmissibility, viral family, host range) and human behavior (e.g., interaction with other species, invasion of habitats) also affect the spillover risk. The dose and route of human exposure to pathogens also determine the chances of a pathogen crossing the species barrier. After a pathogen successfully reaches a new host, other factors will affect the outcome of the spillover. Not all spillover events result in an epidemic outbreak, and many spillover events go unnoticed, without medical or epidemiological importance. An outbreak or epidemic only occurs when the pathogen, after crossing the barriers between species, finds favorable conditions for its dissemination in the new population. These conditions are usually population agglomeration, unplanned urbanization, and a large number of susceptible hosts (Plowright et al. 2017, Becker et al. 2019, Borremans et al. 2019, Ellwanger & Chies 2021, Grange et al. 2021, Nandi & Allen 2021).

The human immunological status at the time of contact with a new zoonotic pathogen influences the outcome of a spillover event. Immunosuppressed individuals can be infected by viruses, fungi, parasites, and bacteria much more easily than individuals with fully competent immune systems (Raychaudhuri et al. 2009, Vanichanan et al. 2018). Both the maintenance of a pandemic status and the raising of new pathogenic variants are conditions affected by the human immunological status, as can be seen in the current COVID-19 pandemic dynamics. Similarly, HLA alleles and variants in immune-system genes (e.g., single nucleotide polymorphisms in Toll-like receptor, cytokine and chemokine receptor genes, complement system) can either increase or decrease the

risk of infection by different pathogens in human populations, in addition to affecting the progression of infection and the host's pathogen load, and, consequently, disease spread in the population (Burgner et al. 2006, Chang et al. 2008, Pine et al. 2009, Ferguson et al. 2011, Adriani et al. 2013, van den Broek et al. 2020, de Vries et al. 2020, Sánchez-Luquez et al. 2021), indicating the importance of host genetics as a determinant of spillover risk and outcome. Therefore, this information makes it clear that when a new pathogen reaches a human being due to favorable ecological conditions (e.g., contact between species sharing the same habitat, land-use changes), the outcome of the spillover event will also be conditioned to a series of other biological factors.

## **CONNECTIONS BETWEEN ENVIRONMENTAL DISTURBANCES AND ZOO NOTIC SPILLOVER**

### **Human behavior and demography**

Human behavior and demographic changes are critical modulators of risk and outcome of spillover events. Keeping animals in captivity for decorative or entertainment purposes, the frequent and close contact with wild species, as well as human entry into wild environments, facilitate spillover events because they put humans in close contact with different species. For example, tourist activities involving cave exploration in Africa facilitated Marburg infection cases in past years. Caves are usually visited by numerous animals, including fruit bats (*Rousettus aegyptiacus*) that act as Marburg reservoir hosts; caves are places where animal defecate and associated pathogens are found in abundance (Johnson et al. 1996, CDC 2009, Amman et al. 2012). Also related to human behavior, the use of wild or exotic animals as pets can facilitate the introduction of new

pathogens into the human population (Chomel et al. 2007), in addition to being a conservation problem affecting wild species.

Human migratory flows can also change the epidemiology of infectious diseases through the introduction of known and unknown pathogens into new areas, by overburdening health systems, or by exposing non-vaccinated migrants to new pathogens and precarious health conditions. These problems are particularly important in cases of forced migration due to war, political instability and climate change. This indicates that the global political instability associated with the disparity in terms of access to healthcare directly or indirectly affects populations worldwide concerning control and prevention of infectious diseases (Gushulak & MacPherson 2004, Castelli & Sulis 2017, Berry et al. 2020, Ibáñez et al. 2021). Recent measles outbreaks in Brazil and Colombia due to Venezuelan migration demonstrated failures in the vaccination and access to health services by Venezuelans (Hotez et al. 2020). The number of 'climate refugees' will increase as climate change intensifies, contributing to both the exposure of migrants to new reservoir hosts and related pathogens, and to the change in the profile of infectious diseases in many countries (McMichael 2015). However, we stress that the effect of migratory flows on infectious disease burden on migrants and refugees is greater than the effect on the population of the country that receives the immigrants, especially in Europe (Castelli & Sulis 2017).

### **Exploitation of wildlife**

Hunting, wildlife trafficking, animal trade in 'wet markets,' and 'bushmeat' consumption are classic driving forces of spillover events, since these practices put humans in close contact with pathogens in the meat, blood and other biofluids from a wide range of animal species (Karesh et al. 2005, Smith et al. 2012, Johnson et al. 2020,



Magouras et al. 2020, Zhang & Holmes 2020). The expression 'wet markets' refers to places where different live animal species are sold in close contact, sometimes sharing a same cage. Some wet markets sell endangered species (Zhang & Holmes 2020, Peros et al. 2021). Wet markets are frequently associated with bushmeat. The expression 'bushmeat' refers to the meat of hunted wild animals sold in popular or wet markets (Pangau-Adam et al. 2012), in some situations in the absence of adequate sanitary standards according to regulatory agencies (Naguib et al. 2021, Peros et al. 2021, Saylor et al. 2021, WHO 2021). Sanitary requirements may vary depending on the sanitary regulations of each country, and traditional food markets can be considered safe when operating in accordance with health regulations (WHO 2021).

Different from 'bushmeat' (hunted meat for income purposes), 'wild meat' refers to the meat of wild animals killed for consumption by hunters and their families. As bushmeat products usually come from systematic hunting activities, including frequent handling of animal carcasses, blood and viscera without sanitary control or inspection, bushmeat is associated with increased spillover risk (Wolfe et al. 2005, Pangau-Adam et al. 2012, Peros et al. 2021). For these reasons, wet markets and bushmeat consumption are recognized as important drivers of zoonotic spillover, unlike markets or fairs where meat products are sold under sanitary inspection, which reduces the risk of transmission of pathogens to humans (Wolfe et al. 2005, Karesh & Noble 2009, Zhang & Holmes 2020, Naguib et al. 2021, Peros et al. 2021). In these places, spillover risk also exists [as indicated by human outbreaks of food-borne diseases in high-income nations like the UK (Public Health England 2018)], but the risk is lower due to sanitary control.

### **Land-use changes and exploitation of Indigenous lands**

Other human-mediated activities also facilitate spillover events, including deforestation, industrial livestock, monoculture farming, and mining, among other types of human alterations on land. These changes are commonly unified in the expression 'land-use changes.' Land-use changes lead to host exposure to a new array of pathogens (Murray & Daszak 2013). The construction of roads in wild landscapes (e.g., Amazon rainforest), besides causing damage to ecosystems (Ferrante & Fearnside 2020a), increases the contact of humans with forest-associated animal species and the risk of spillover. Although the human presence can scare away some animal species, when humans invade forest environments to build roads or to perform mining and logging activities, among other reasons, the contact with animal species increases, especially contact with mosquitoes and other blood-sucking insects that benefit from the human presence that provides an additional food source. This closer and more frequent interaction between humans and anthropophilic insects favors spillover events mediated by invertebrate intermediate hosts (Ellwanger et al. 2020).

Extensive land-use changes and associated spillover risk are also a major issue for Indigenous populations. Due to limited contact with non-Indigenous populations, Indigenous peoples have weak or no natural/protective immunity to pathogens that emerged outside Indigenous areas. Such populations also have limited access to vaccines and healthcare facilities. These factors exacerbate the burden related to emerging pathogens in Indigenous populations. This is a problem observed in several situations and in various parts of the world, from the colonization of the Americas and Africa by Europeans to the ongoing COVID-19 pandemic

in Brazil, among other situations (Valeggia & Snodgrass 2015, Ferrante & Fearnside 2020b). Of particular concern is a proposed law in Brazil (PL191/2020) opening Indigenous lands to mining, logging, agriculture and other activities by non-indigenous people (Villén-Pérez et al. 2021). The risk is clear in a project that is already moving ahead to grow corn (maize) to feed pigs in an Indigenous area in association with a food and biofuel company (Ferrante et al. 2021). Land-use activities in the Indigenous areas expose both Indigenous peoples and workers to a new range of potential exotic pathogens.

Similarly, human contact with other animal species is facilitated by habitat fragmentation (Wilkinson et al. 2018). For example, the transmission of zoonotic parasitic diseases such as leishmaniasis and Chagas disease is facilitated in areas with fragmented vegetation due to the increased human contact with the vectors of *Leishmania* and *Trypanosoma* parasites (phlebotomine sandflies and triatomine bugs, respectively), and changes in the composition and infectious status of wild hosts (Vaz et al. 2007, Roque et al. 2008, Curi et al. 2014, Zaidi et al. 2017, Cardozo et al. 2021). In a general sense, the maintenance of habitat core/solidity reduces the habitat perimeter, diminishing the human contact with other species and, consequently, the spillover risk. On the other hand, habitat fragmentation increases the habitat perimeter and contact zones where pathogen transmissions may occur between non-human animals and humans (Wilkinson et al. 2018, Borremans et al. 2019, Bloomfield et al. 2020). Specifically, there are examples showing that habitat fragmentation in Africa was associated with increased human contact with non-human primates, bats, and potentially the zoonotic pathogens found in these animals (Rulli et al. 2017, Bloomfield et al. 2020). A recent study reported that the risk of SARS-related

coronavirus outbreaks in China is higher in areas with forest fragmentation and concentrations of livestock and humans (Rulli et al. 2021). Habitat fragmentation is strongly associated with loss of ecosystem functions, reduced landscape connectivity, and biodiversity loss (Haddad et al. 2015), which impairs the dilution effect and increases the risk of zoonotic diseases through this additional mechanism (Allan et al. 2003, Keesing et al. 2006). These factors act in synergy with the proliferation of species adapted to human-modified environments and an increase in the load of pathogens hosted by these species, thus creating favorable conditions for the transmission of relatively new zoonotic pathogens to humans.

### **Livestock industry and antimicrobial resistance**

The large scale of the livestock industry for the production of meat and other animal products leads to the confinement of a large number of animals in small areas, usually with frequent contact with humans and other species. As previously described, environments with low species richness can limit the dilution effect, favoring the spread of pathogens. In addition, the movement of livestock within and between countries with little or no sanitary inspection poses a threat to the dissemination of infectious diseases if these animals carry pathogens with zoonotic potential, such as Rift Valley fever virus, as seen in East-African countries that export livestock (Anyamba et al. 2001, Martin et al. 2008, Taylor et al. 2016).

Animals from livestock production also act as intermediate hosts for the adaptation of pathogens from wildlife before they are introduced into the human population. For example, swine (e.g., domestic pigs) are considered to be 'mixing vessels' where strains of influenza A viruses from wild birds can undergo



genetic recombination or reassortment with other viruses present in pigs, originating new influenza strains that will then be transmitted to the human population. This occurs because pigs have cell receptors recognized by avian and human influenza viruses, in addition to sharing the environment with different species of birds and humans (Ma et al. 2008, Ellwanger & Chies 2021). The role of pigs as mixing vessels for the reassortment of influenza viruses has already been shown by various studies, confirming that pigs can act as intermediate hosts for the adaptation of animal influenza viruses before being introduced into the human population (Zhou et al. 1999, Urbaniak et al. 2017, Zell et al. 2020). In a study performed in Egypt, Gomaa et al. (2018) found evidence of infection with avian (H9N2, H5N1), human (pandemic H1N1), and swine influenza viruses in pigs. Ganti et al. (2021) recently showed that mallard ducks also have the potential to act as mixing vessels for the reassortment of influenza A viruses.

Animals from livestock production (e.g., cattle, swine, poultry) also pose a zoonotic risk to human populations considering diseases caused by parasites, especially when these animals are raised in inadequate facilities and with poor hygiene conditions. Infection by *Fasciola hepatica*, *Schistosoma japonicum*, *Trichinella spiralis*, among other parasitic infections, can affect humans due to problems in the practices of breeding, confinement and sanitary inspection of livestock animals and derived products (Gortázar et al. 2007, Rist et al. 2015). Livestock can act as bridges (intermediate hosts) for the transmission of parasites from wild hosts to humans (Gortázar et al. 2007, Wiethoelter et al. 2015).

Finally, it is possible that the introduction (spillover) of SARS-related viruses (SARS-CoV, MERS-CoV) to the human population from bats, source hosts for both SARS-related viruses,

has the participation of intermediate hosts, specifically palm civets for SARS-CoV and camels for MERS-CoV. However, the direct bat-human transmission of these viruses cannot be ruled out (Letko et al. 2020). Some farmed species such as minks, red foxes, and raccoon dogs, potentially acted as intermediary hosts in the SARS-CoV-2 spillover into the human population, but this represents an open question (Koopmans et al. 2021, Lytras et al. 2021). The large number of animals observed in industrial livestock production and the frequent contact with other animal species create numerous opportunities for the adaptation of new pathogens before reaching the human population.

The intensive use of antimicrobial drugs in the livestock industry creates ideal conditions for the selection of microorganisms resistant to multiple drugs and for the emergence of new pathogenic microbial strains, reinforcing opportunities for spillover events (Ye et al. 2016, He et al. 2020, Magouras et al. 2020). Drug-resistant pathogens were responsible for ~20% of all emerging infectious-disease events reported since 1940, a phenomenon stemming from the pervasive use of antimicrobial drugs (Jones et al. 2008). Inappropriate intensive use of antimicrobials in human medicine (e.g., azithromycin as a supposed COVID-19 treatment) will contribute to the emergence of multiresistant strains (Afshinnkoo et al. 2021). The role of drug resistance in the emergence of outbreaks and epidemics is expected to gain greater attention in the coming decades, along with anthropogenic pressures on the environment and animal species.

### **Fires and other drivers of unusual movement pattern of animals**

Fires, deforestation, and habitat loss induce wild animals to assume unusual movement patterns and alternative spatial distributions because

these animals need to leave their natural habitats to obtain food, water and shelter, or to escape fire, among other reasons (Johnson et al. 1992, Hadley & Betts 2009, Niebuhr et al. 2015, Nimmo et al. 2019, Ramos et al. 2020). In response to such events, animals often supply their needs in forest-city borders and in urban and peri-urban areas (e.g., migration of non-human primates from wild areas to cities), especially when urban settings are established in areas previously occupied by forests. For instance, in the Brazilian cities of Rio de Janeiro and Porto Alegre, non-human primates share forest fragments with the human population (Cunha et al. 2006, Corrêa et al. 2018). Also in Brazil, non-human primates (howler monkeys) and forest-dwelling mosquitoes found in city-forest interfaces (as a consequence of urbanization, habitat loss and forest fragmentation) can act as bridges between the sylvatic and urban cycles of yellow fever, as well as bridges for the spillover of new human pathogens from wildlife (Cardoso et al. 2010, Almeida et al. 2012, Couto-Lima et al. 2017).

In addition to inducing animals to explore new environments due to habitat loss, fires can favor the occurrence of arboviral diseases. In Brazil, studies have associated fires with outbreaks of Dengue, Zika, Chikungunya and Yellow fever, especially in areas where fires have an anthropogenic origin and are associated with the expansion of agriculture and livestock production (Torres et al. 2019, Moreno et al. 2021). The increase in fire outbreaks recently observed in Brazil and other countries (Pivello et al. 2021) will potentially increase the risk of spillover events involving arboviruses.

Animal trafficking and the domestication of wild animals also contribute to changes in the geographical distribution of animal species and pathogens with zoonotic potential. These processes can put human populations into contact with new pathogens from exotic animals

that have been artificially moved to new areas and environments. For example, zoonotic *Salmonella* outbreaks were associated to animal trafficking and exotic pets (e.g., Amazon parrots) (Marietto-Gonçalves et al. 2010, Saidenberg et al. 2021). Also, Kovalev & Mazurina (2022) recently evaluated Omsk hemorrhagic fever, an endemic disease from Western Siberia and associated with muskrats (*Ondatra zibethicus*). Since the Omsk hemorrhagic fever virus (OHFV) is closely related to the tick-borne encephalitis virus (TBEV), considering genetic and ecological characteristics, the authors suggested that the OHFV originated directly from the TBEV (Far Eastern subtype) in a spillover event involving the transmission of the virus from *Ixodes persulcatus* ticks to muskrats after the human introduction of *O. zibethicus* to Western Siberia in the second half of the 1930s. The introduction of *O. zibethicus* in this new region was motivated by the potential use of muskrat's valuable fur (Kovalev & Mazurina 2022).

Unusual animal movement patterns are also of epidemiological concern when they involve domestic animals with competence for the transmission of zoonoses, potentially increasing the risks of zoonotic spillover or creating conditions for these animals to act as bridges to pathogen hosts. Dogs that circulate between urban and forest areas can facilitate the spillover and spillback (human-to-animal transmission) of many pathogens, increasing the infectious-disease risk for both human and animal populations (Martinez et al. 2013, Ellwanger & Chies 2019).

### **Biotic and abiotic environmental changes**

Studies performed with mosquitoes are critical to comprehend how human disturbance of the environment can lead to an increased risk of spillover events mediated by vectors (as intermediary hosts). Environments with high

biodiversity tend to have a greater variety and abundance of predators of disease vectors. These predators include bats, birds, amphibians and larvivorous fishes that feed on mosquitoes at different stages of development. Reduction of the diversity of predators due to anthropic action can benefit the survival and proliferation of mosquitoes. Also, abiotic factors (e.g., sunlight, wind patterns, temperature, moisture, and the pH of water in breeding sites) are altered in degraded landscapes and can affect vector distribution and proliferation (Burkett-Cadena & Vittor 2018, Almeida et al. 2019, Franklinos et al. 2019). For example, lower temperatures in the forest can slow the larval development of mosquitoes while the opposite can occur when forests are cleared, resulting in warmer temperatures, greater light intensities, and increased availability of nutrients in water pools, thus benefitting the larvae of some mosquito species (Burkett-Cadena & Vittor 2018, Franklinos et al. 2019). Consequently, these ecological and abiotic changes benefit mosquito populations and increase the risk of spillover events mediated by vectors (Burkett-Cadena & Vittor 2018, Ellwanger & Chies 2018, Almeida et al. 2019, Franklinos et al. 2019). From a global perspective, it is very likely that climate change in the coming decades, including a 1.0–3.5°C increase of global temperature and more frequent climatic anomalies (e.g., El Niño, droughts, floods), will lead to an increased burden of vector-borne diseases and more zoonotic spillover events mediated by arthropod vectors (Githeko et al. 2000, Watts et al. 2019, Wilke et al. 2019b).

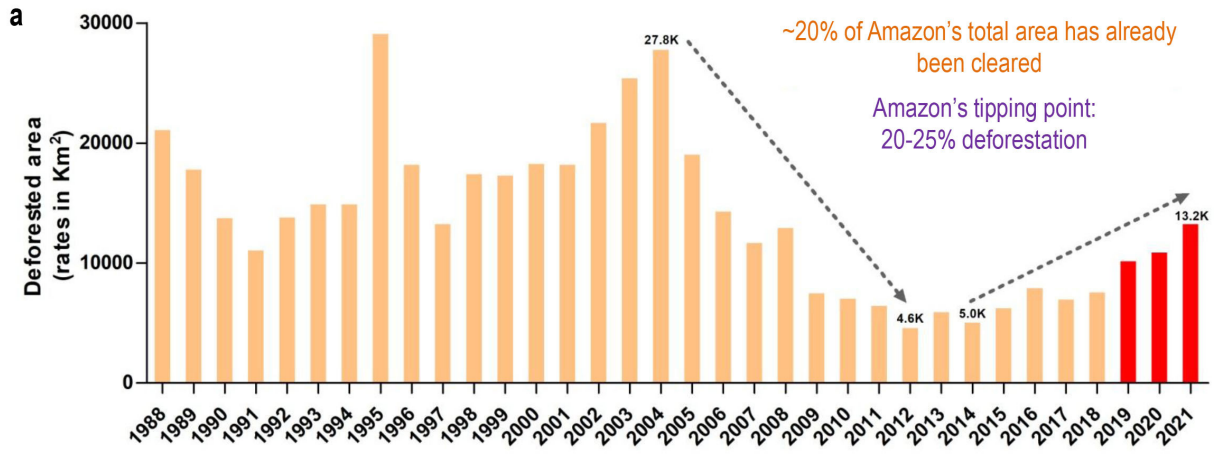
Finally, it is essential to consider that disease cycles are often complex, being influenced by factors that go beyond the abiotic sphere, such as biological aspects of vertebrate hosts (immunity, genetics, and other characteristics, as discussed previously), arthropod resistance to insecticides, and stresses on communities of vectors (Guedes et al. 2017, Pavlidi et al. 2018).

Therefore, the impact of climate change on vector-borne and other zoonotic diseases will be affected by these other factors, making it difficult to accurately predict the intensity of impacts and distribution of pathogens and diseases in a changing world. Considering these uncertainties, the precautionary principle must be considered (Mahrenholz 2008) and anthropogenic changes in the environment must be controlled in order to reduce zoonotic risks to the human population.

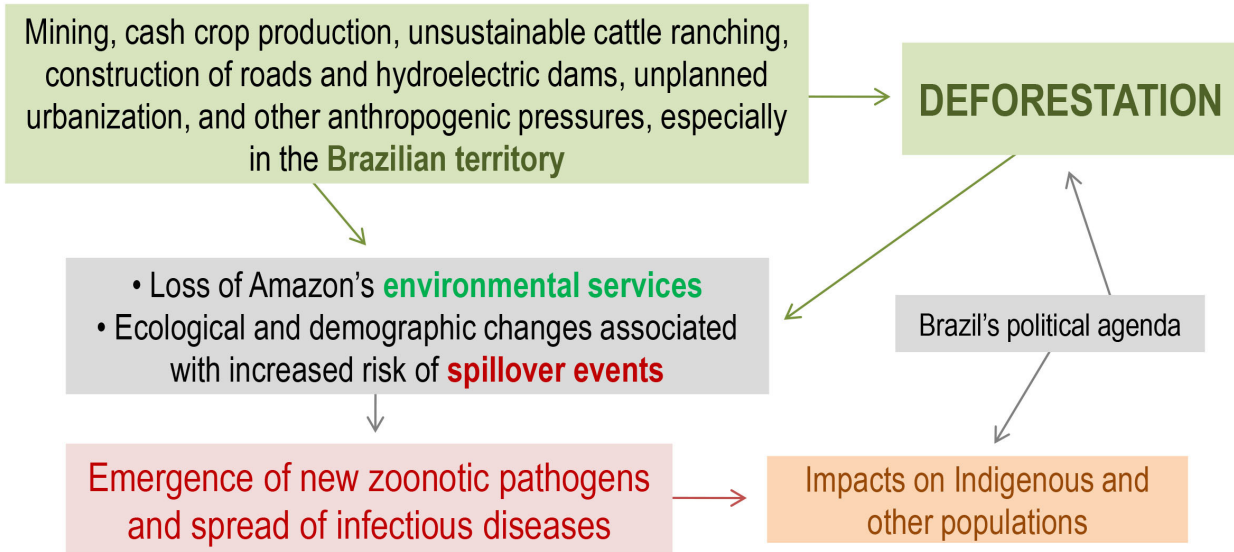
### **ANTHROPOGENIC ACTIVITIES IN THE AMAZON REGION AND THEIR POTENTIAL IMPACTS ON SPILLOVER EVENTS**

The Amazon Forest is one of the most biodiverse regions in the world, with 70% of the Amazon basin located within Brazil (Kirby et al. 2006). Due to its high biodiversity combined with a diversity of anthropogenic activities in the region, the Amazon Forest is a hotspot for the emergence of new pathogens (Val 2020). Indeed, there are numerous potential new pathogens in the Amazon Forest that could pose a risk to human populations. However, it is the intense human activity in the region that is the main driver of potential spillover events in the Amazon Forest, not the biodiversity *per se*.

Degradation of tropical forests, including the Amazon Forest, is strongly derived from economic activities linked to the exploitation of minerals, oil, and timber, in addition to industrial livestock and monoculture production. Globalization and economic connections between developed and developing countries mean that the triggers of environmental degradation in any given part of the world can be derived from demands of distant countries or even other continents. For instance, land-use changes in the Amazon Forest, including the increasing deforestation rate in the region (see Figure 1, panel a, for more data), are partially triggered by the demand for



**b Amazon Forest: a biome with high biodiversity**



**Figure 1.** Deforestation rate in Brazilian Amazon Forest (Legal Amazon) between 1988 and 2021 and connections between anthropogenic pressures on Amazon Forest and spillover risk. Panel a: between 2004 and 2012, deforestation in the Amazon underwent a significant reduction, partly as a result of the strengthening of policies for controlling illegal activities. From 2014 onwards there have been increases in deforestation rates, with alarming results in 2019, 2020 and 2021, reflecting the weakening of the regulation of illegal activities in the region. Tipping point: the point at which the Amazon Forest stops properly providing its environmental services (e.g., hydrological cycle, maintenance of carbon stocks), losing many rainforest characteristics and enters into a self-perpetuating decline. Data (deforestation rate by year collected on February 1st, 2022) obtained from TerraBrasilis - *Programa de Cálculo do Desflorestamento da Amazônia* (PRODES), Instituto Nacional de Pesquisas Espaciais (INPE); data under CC BY-SA 4.0 license (INPE 2022). The graph was plotted using GraphPad Prism. Additional information was obtained from Aguiar et al. (2016), Lovejoy & Nobre (2018), and Ferrante & Fearnside (2019). Panel b: deforestation and other anthropogenic pressures on Amazon Forest are closely connected activities. These pressures facilitate spillover events, the emergence of pathogens and the spread of infectious diseases, affecting populations living inside and outside the Amazon region.

beef and agricultural commodities by China and European countries (Fearnside et al. 2013, Fuchs et al. 2019, Pendrill et al. 2019), by the bovine leather industry in Europe (Mammadova et al. 2020), among other economic drivers. These human activities in the Amazon region facilitate the risk of zoonotic spillover events and the spread of infectious diseases in multiple ways (Figure 1, panel b).

A recent study performed in the Amazon rainforest showed that anthropogenic pressure on the natural landscape, specifically forest fragmentation, decreases mosquito diversity and increases the abundance of malaria vectors such as *Anopheles (Nyssorhynchus) darlingi* mosquitoes (Chaves et al. 2021). Human occupation in forest areas causes loss and fragmentation of habitat. In association with this, there is an increase in the availability of human hosts and a blockage of water flow, thus facilitating the dispersion and proliferation of human-associated mosquito species with medical importance, like *An. (Ny.) darlingi* (Chaves et al. 2021). These data reinforce the concept that diversity of species is important for the prevention of vector-borne diseases. Furthermore, the same study (Chaves et al. 2021) demonstrates that anthropogenic actions favor the abundance of medically important mosquitoes not only in urban environments but also in tropical forests. In accordance with the information described above, Prist et al. (2022) recently showed that the construction of roads and the associated increase in forest fragmentation and forest edges facilitate yellow fever virus dispersion. Road construction and associated environmental degradation have been a threat to the Amazon biome from the 1970s to the present (Barni et al. 2015, Ferrante & Fearnside 2020a).

The construction of hydroelectric dams in tropical forest areas can result in population

explosions of some mosquito species, as occurred in Brazil's Tucuruí Dam for *Mansonia* species (Tadei et al. 1991, Fearnside 1999) and at the Samuel Dam for *Culex* species (Fearnside 2005). In the first years after dam construction, large areas of the reservoirs were covered by aquatic plants (macrophytes) that provide breeding grounds for *Mansonia* mosquitoes (Fearnside 2001). Potential spillover events involving mosquito-borne pathogens are therefore a major concern in the Amazon region.

Hunting and commercialization of wild animals in the Amazon region is very intense, with a bushmeat market reaching up to 6.49 kg per person/year in the central Amazon (quantity varies by Amazon region) (van Vliet et al. 2014, El Bizri et al., 2020). These data suggest that spillover events derived from bushmeat practices are a recurrent possibility in the Amazon region, similar to what happens in other countries with high biodiversity (Ellwanger & Chies 2021).

In addition to habitat fragmentation, construction of water reservoirs and bushmeat practices, logging, mining, and other exploitative (and often illegal) economic activities in the Amazon region trigger a number of ecological and demographic changes, including migratory flows, habitat loss, unplanned urbanization, prostitution, pollution, climate change, and extreme weather events (Ellwanger et al. 2020). As discussed earlier in this article, these conditions directly or indirectly favor the occurrence of spillover events and the spread of emerging pathogens. For this reason, deforestation in the Amazon Forest and other anthropogenic activities in the region create the "perfect storm" of infectious diseases in the Amazon region (reviewed in Ellwanger et al. 2020).

The potential emergence of pathogens in the Amazon Forest may affect populations living in the region as well as people living outside the Amazon region. As exemplified by the COVID-19



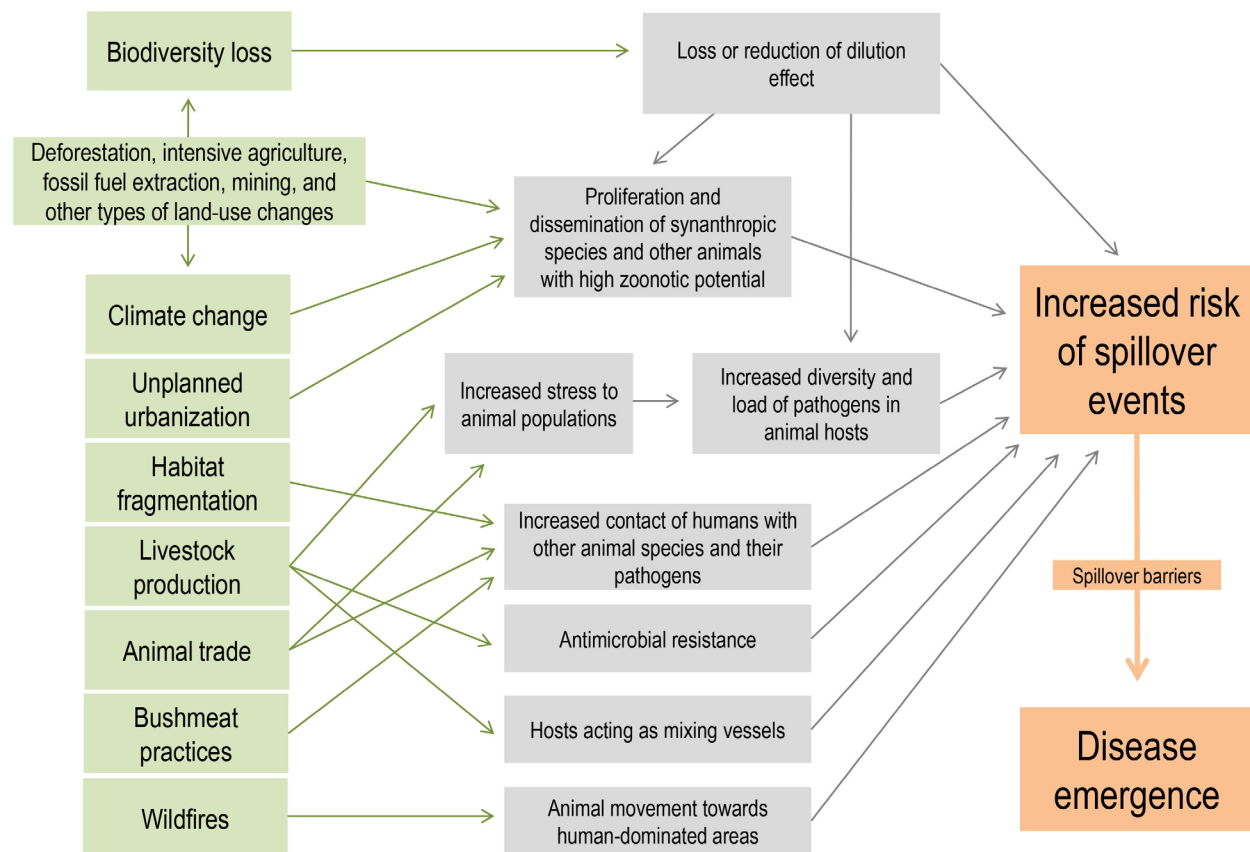
pandemic, emerging pathogens can spread across the world very easily and quickly. Thus, conserving the Amazon Forest is critical not only to protect biodiversity and associated ecosystem services (e.g., water cycling, carbon stock maintenance); protecting the Amazon biome is a global public health measure (Fearnside 2008, Ellwanger et al., 2020).

Combating deforestation, mining and other types of land-use change is difficult to achieve, but this is not an impossible task. In Brazil, deforestation in the Amazon region declined greatly (~70%) between 2005 and 2012 (Figure 1, panel a), in part due to government policies (West et al. 2019, Dobson et al. 2020, West & Fearnside 2021). These policies have ended under Brazil's presidential administration that took office in January 2019 (Ferrante & Fearnside 2019, 2020b), but the potential for controlling deforestation through government policies remains an essential lesson. A recent study (Dobson et al. 2020) pointed out that the costs of mitigating a pandemic such as the COVID-19 are much greater (estimated at US\$8.1 to US\$15.8 trillion) than the amount that would have to be invested to prevent the main drivers of emerging infectious disease events, estimated at US\$17.7 to US\$26.9 billion per year. Of note, the prevention costs for 10 years would represent ~2% of the costs of the COVID-19 pandemic (Dobson et al. 2020). In other words, conservation actions protect the environment, limit the spread of infectious diseases, and are cheaper than bearing the burden of emerging infectious disease events. Also, in Brazil, the demarcation of Indigenous lands (*Terras Indígenas*) is an effective way of limiting the exploitation of natural resources and land-use changes while protecting traditional communities. The contribution of protected areas governed by local communities and Indigenous peoples in the field of biodiversity conservation is widely recognized (Corrigan et al.

2018). Considering that Brazil holds most of the territory of the Amazon Forest and has strong political and economic powers, the country needs to take the lead in the conservation of the region, contributing to the reduction of the risks of potential spillover events in the Amazon Forest.

## CONCLUSION

This article synthesized the main connections between human-related environmental disturbances, ecological modifications, and increased risk of spillover events (Figure 2), primarily based on examples and models from different world regions. In brief, anthropogenic disturbances in the environment lead to changes in ecological niches, reduction of the dilution effect, increased contact between humans and other animals, changes in the incidence and load of pathogens in animal populations, and alterations in the abiotic factors of landscapes, among other ecological changes. These alterations can increase the risk of spillover events, facilitating new infectious disease outbreaks. In addition to our interpretation of the issues addressed in this paper, we emphasize that other models also explain the relationship between human activity, environmental disturbances, and emerging infectious diseases (e.g., Wolfe et al. 2007, Parrish et al. 2008, Karesh et al. 2012, Morse et al. 2012, Murray & Daszak 2013, Faust et al. 2018, Glidden et al. 2021). These interpretations are generally not mutually exclusive, and in most cases are complementary. Above all, it is important to keep in mind that generalist explanations for the emergence of infectious diseases will always be incomplete (Jones et al. 2013). Each outbreak, epidemic, pandemic, or small-scale zoonotic event has its specific characteristics and triggers that are inherent to the place and



**Figure 2.** Connections between human-related environmental disturbances, ecological modifications and increased risk of zoonotic spillover events. Spillover barriers are factors that facilitate or hinder the transmission of pathogens between different species/populations. They can be biological (e.g., genetic proximity between hosts, immunological and genetic factors), demographic (e.g., overcrowding), ecological (e.g., habitat sharing), cultural (e.g., bushmeat practices), and associated with pathogens (e.g., virulence, survival in the environment). These barriers affect both spillover risk and the outcome of spillover events. More information concerning spillover barriers can be found in Plowright et al. (2017) and Ellwanger & Chies (2021).

context in which it occurred. Considering the specificities of each environment, our review brought a discussion specifically focused on the Amazon rainforest, showing that increasing anthropogenic damage in the region may also increase the risk of zoonotic spillover events and spread of infectious diseases, impacting the Amazon populations and potentially populations elsewhere (Figure 1). Finally, conservation efforts lead to benefits to different global spheres in an integrated manner, as they help to contain anthropic activities on the environment and to reduce the risk of zoonotic spillover events.

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JHE wrote the initial version of the article and coordinated the work. PMF, MZ, JMVV, ABGV, GFV, EB, JCC, FNDM, GL, LC, BKL, VLK, ESS, FRS, MNW, SEMA, VPH, JABC contributed with opinions on the content of the article, examples, and writing/editing the text. JABC supervised the work. All authors approved the article.



# A saúde humana e ambiental depende do Pampa

**Artigo | Joel Henrique Ellwanger, Marina Ziliotto e José Artur Bogo Chies, pesquisadores nas áreas de genética e ecologia de doenças, defendem que a proteção do bioma pode prevenir doenças emergentes e promover a saúde física e mental**

*\*Por: Joel Henrique Ellwanger, Marina Ziliotto e José Artur Bogo Chies*

*\*Foto: Alexandre Copês*

O Brasil é internacionalmente conhecido pela imensa riqueza natural, sendo a imagem do país, no imaginário popular, fortemente associada à vastidão e diversidade de suas florestas. De fato a biodiversidade dos biomas florestais, Amazônia e Mata Atlântica, é facilmente detectada em decorrência da exuberância da vegetação. Nossos biomas não florestais (Cerrado, Caatinga, Pantanal e Pampa), porém, também contêm grande biodiversidade, apenas expressa de forma mais sutil do que nas florestas. Por exemplo, um [estudo](#) publicado em 2018 por pesquisadores da UFRGS registrou 56 espécies vegetais em uma área de apenas 1m<sup>2</sup> do Pampa.

*Apesar da identificação dos gaúchos com as tradições e os costumes popularmente associados ao Pampa, pouca atenção está voltada à [preservação de sua biodiversidade](#).*

Apenas [1% do Pampa está sob proteção estrita](#), e dados recentes divulgados pelo [MapBiomas](#) confirmam que esse importante bioma está extremamente ameaçado, com um aumento de 92% na perda de sua vegetação no período entre 2020 e 2021. Essa perda está vinculada principalmente à conversão da vegetação natural em pastagens plantadas e em áreas destinadas ao cultivo de monoculturas, como arroz e soja. Mesmo a criação de gado, atividade compatível com o Pampa, pode se tornar prejudicial ao bioma no caso de excesso de animais. Outros fatores também contribuem para a degradação do Pampa, incluindo a ocupação do bioma de forma desordenada, a poluição e a mineração.

A perda do Pampa é preocupante porque prejudica [serviços ecossistêmicos](#) fundamentais para a vida de humanos e de espécies selvagens, como a polinização, a captura de dióxido de carbono (CO<sub>2</sub>) e a regulação do clima e dos ciclos da água. Em [carta recentemente publicada na revista Science](#), destacamos a importância da biodiversidade do Pampa para a manutenção da saúde humana e ambiental. Destacamos a seguir três pontos mencionados na publicação: prevenção de zoonoses e doenças emergentes; sistema imune saudável e saúde mental; e redução da poluição.

Em ecologia, existe um conceito conhecido como “[efeito diluição](#)”, significando que em áreas com grande biodiversidade há uma diminuição da “concentração” de animais hospedeiros e de vetores de patógenos devido à quantidade aumentada de espécies. De forma simplificada, a biodiversidade reduz os riscos de transmissão de doenças que podem afetar humanos e animais, as zoonoses. Já ambientes com pouca biodiversidade estão associados a um maior risco de ocorrência de doenças. Além da redução do efeito diluição, a perda da biodiversidade aumenta as chances de surgimento (emergência) de novas doenças através de outros mecanismos. Atividades como remoção da vegetação e caça de animais silvestres facilitam o contato de humanos com outros animais, favorecendo a [transmissão de patógenos entre diferentes espécies](#).

*Ou seja, a preservação do Pampa é fundamental para a prevenção de zoonoses e doenças transmitidas por vetores.*

A perda da vegetação do Pampa, em associação com a pecuária excessiva, contribui para a liberação de CO<sub>2</sub> e metano na atmosfera, promovendo mudanças climáticas, outro fator fortemente associado ao risco aumentado de emergência e à disseminação de doenças infecciosas. Ondas de calor e frio extremo também causam importantes prejuízos à saúde humana, afetando especialmente os idosos. Secas e problemas na produção de alimentos decorrentes das mudanças climáticas impactam a saúde da população pelo empobrecimento econômico e pela redução da segurança alimentar. Essas informações



evidenciam que a preservação do Pampa é essencial para o controle das mudanças climáticas e a redução dos riscos de saúde associados a elas.

O Pampa “clássico” é popularmente associado aos campos de gramíneas observados nas regiões de fronteira com a Argentina e o Uruguai. Isso está correto, porém o bioma também está presente em áreas mais urbanizadas do Rio Grande do Sul, mesmo que de forma mais fragmentada. Porto Alegre, por exemplo, encontra-se geograficamente na transição entre Pampa e Mata Atlântica. Nesses locais mais urbanizados, mas que ainda preservam parte da vegetação natural, interações entre a população gaúcha e o Pampa também ocorrem com alta frequência. Tais interações trazem importantes [benefícios para a saúde](#). Por exemplo, o contato com a natureza contribui para que o sistema imunológico de crianças se desenvolva de forma saudável.

*A interação com um ambiente biodiverso, através do consumo de alimentos variados e do contato com microrganismos naturalmente presentes no ambiente, é importante para a manutenção de uma microbiota saudável, permitindo a incorporação de uma ampla gama de microrganismos benéficos à saúde humana.*

É difícil mensurar exatamente os efeitos do contato com a natureza sobre a redução de estresse e ansiedade. No entanto, é inegável um sentimento de bem-estar e relaxamento após um passeio no campo ou um piquenique. A boa notícia é que não é necessário viajar para entrar em contato com o Pampa. A vegetação do bioma também pode ser apreciada em áreas urbanas, como no [Jardim Botânico de Porto Alegre](#). O contato com a biodiversidade do Pampa promove a saúde mental e o bem-estar da população gaúcha.

O uso inadequado e excessivo de pesticidas, no entanto, causa uma série de problemas à saúde humana, além de contaminar o ambiente e prejudicar espécies nativas, [preocupações importantes no Pampa](#). Incentivar práticas agrícolas sustentáveis, como a agricultura familiar e a agroecologia, é alternativa que contribui para preservar a biodiversidade do bioma e reduzir as áreas de monocultura, altamente dependentes de pesticidas.

*A vegetação do Pampa também favorece a remoção de poluentes atmosféricos e reduz a poluição sonora, funções importantes, principalmente em regiões urbanizadas.*

Proteger o Pampa significa mais do que preservar a identidade cultural dos gaúchos; é uma estratégia crítica para a preservação tanto da saúde humana quanto da ambiental, ambas intimamente ligadas pela perspectiva [One Health](#) (ou Saúde Única). A “[Carta aberta à sociedade gaúcha pela proteção do Pampa](#)”, publicada recentemente pela Coalizão pelo Pampa, traz diretrizes e estratégias práticas para o uso sustentável do bioma. A valorização do bioma Pampa não é apenas uma questão de preservação, mas um assunto essencial ao nosso bem-estar.

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