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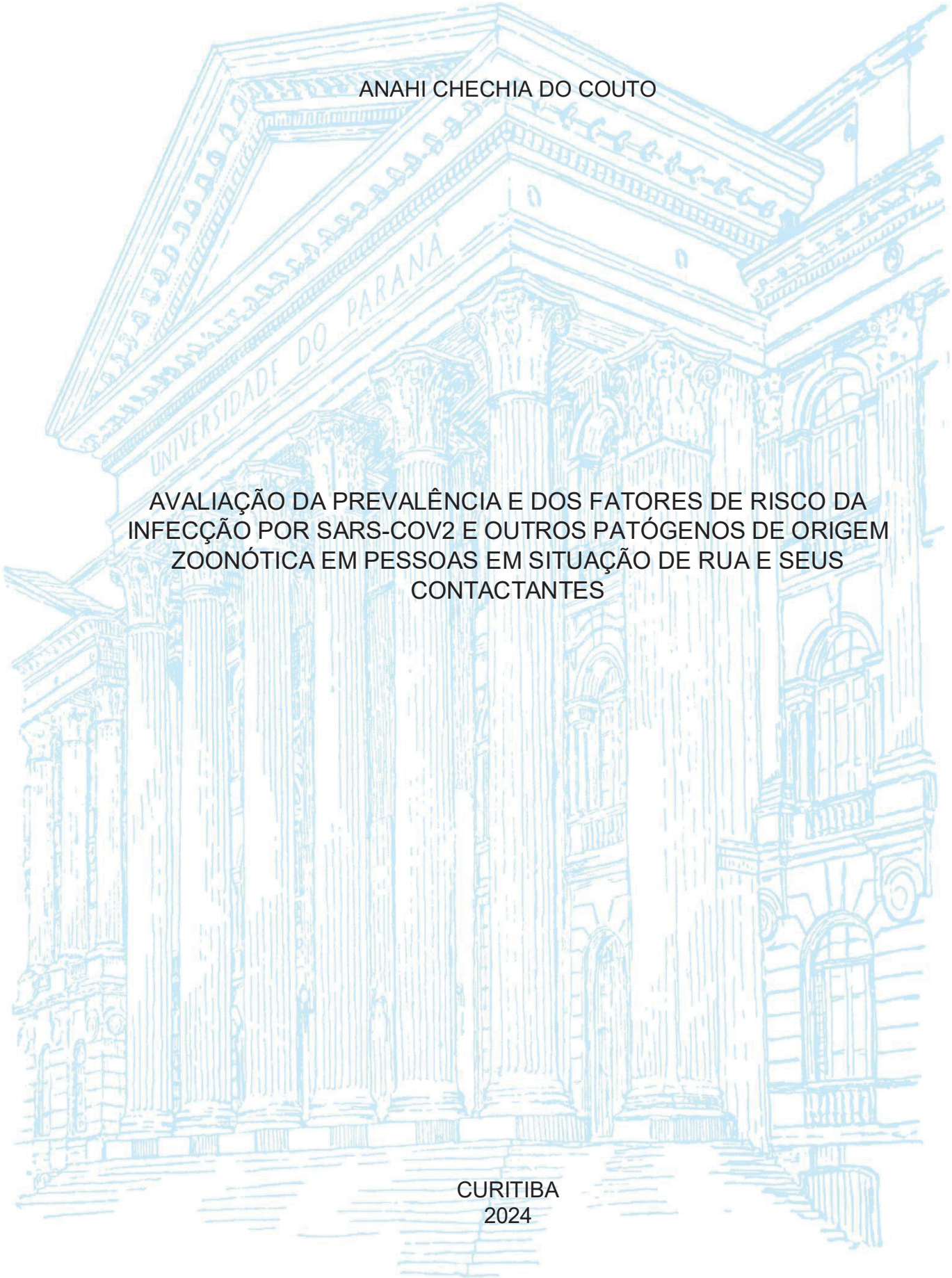

AVALIAÇÃO DA PREVALÊNCIA E DOS FATORES DE RISCO DA INFECÇÃO POR SARS-COV2 E OUTROS PATÓGENOS DE ORIGEM ZOONÓTICA EM PESSOAS EM SITUAÇÃO DE RUA E SEUS CONTACTANTES

CURITIBA
2024

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Tese apresentada ao curso de Pós-Graduação em Biologia Celular e Molecular, Departamento de Biologia Celular, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Biologia Celular e Molecular.

Orientador: Prof Alexander Welker Biondo

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação BIOLOGIA CELULAR E MOLECULAR da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ANAHI CHECHIA DO COUTO** intitulada: **Avaliação da prevalência e dos fatores de risco da infecção por sars-cov2 e outros patógenos de origem zoonótica em pessoas em situação de rua e seus contactantes**, sob orientação do Prof. Dr. ALEXANDER WELKER BIONDO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

Pessoas em situação de rua estão em condição de alta vulnerabilidade social, econômica e sanitária. Sua condição de vida causa além de uma alta mortalidade, uma morbidade maior para diversas doeças e agravos, comparando com a população geral. Com isso esse trabalho levantou a preocupação e o questionamento sobre a exposição à patógenos de origem zoonótica para essa população e seus contactantes. Para avaliar prevalências e fatores de risco de zoonoses nessa população foram desenvolvidos três artigos: o artigo do Capítulo 1, denominado ":High *SARS-CoV-2* seroprevalence in persons experiencing homelessness and shelter workers from a day-shelter in São Paulo, Brazil" investigou a prevalência e os fatores de risco para *SARS-CoV-2* em pessoas em situação de rua que frequentam um centro comunitário e funcionários desse local. Foi realizado uma coleta de material biológico e questionário epidemiológico em um Centro comunitário em agosto de 2020 na cidade de São Paulo. Essas amostras coletadas foram enviadas para laboratório e processadas, realizando teste RT-PCR e ELISA para *SARS-CoV-2*. Todas as amostras de swab foram negativas por RT-qPCR. A soropositividade IgG foi de 111/203 (54,7%) em pessoas em situação de rua, 41/87 (47,1%) em funcionários, respetivamente. Os fatores de risco foram foram "idade acima de 30 anos" para pessoas em situação de rua e "auto-declaração como não-branco" e perda de olfato e paladar" para os funcionários. Já a variavel "ter cão como animal de companhia foi um fator de proteção para pessoasem situação de rua. O Capítulo 2 também usou as mesmas amostras coletadas em 2020, mas para avaliar a soroprevalência de *Toxocara spp.* na mesma população. Com isso o resultado da prevalência foi de 89/194 (45,9%,) em pessoas em situação de rua e 22/79 (27.8%, 95% CI: 19.2–38.6) em funcionários. Ter um grau universitário foi o único fator de proteção para a infeção por *Toxocara spp.*. O terceiro e último capítulo apresenta o artigo: "One health approach on serosurvey of anti-*Leptospira spp.*. in homeless persons and their dogs in South Brazil", que diferente dos anteriores avaliou amostras coletadas em três cidades (São Paulo, Curitiba e Foz do Iguaçu). Um total de 200 amostras humanas e 75 de cães foram testadas para anticorpo anti-*Leptospira spp*. Nenhuma amostra humana foi positiva, enquanto 5 (6,7%) dos cães foram positivos. Os três trabalhos também levantaram o perfil sociodemográfico da população avaliada que indicou dados semelhantes a outros trabalhos que também avaliam essa população, mostrando que a maior parte dessas pessoas são homens com idade entre 20 e 50 anos, se auto-declaram pretos ou pardos e que possuem baixa escolaridade. Portanto, o conjunto de artigos que compõe este trabalho, demonstra que as pessoas em situação de rua e os funconários do centro de acolhimento aqui avaliadas, estão mais expostas à transmissão de COVID-19 do que a população geral. Essas duas populações também tiveram contato com o *Toxocara spp.*, indicando relação com sua vulnerabilidade socioambiental. Já o convívio com cães não foi um fator de risco para a transmissão da leptospirose e o resultado negativo em todas as amostras supostamente pode ser explicado por uma característica comportamental de constante migração, evitando assim o contato com enchentes e alagamentos. Essa tese aponta a vulnerabilidade de pessoas em situação de rua diante da exposição à patógenos de origem zoonotica, mostrando a importância e necessidade de uma maior atenção para com essa população.

Palavras-chave: Vulnerabilidade; SARS-COV2; Toxocaríase; Leptospirose, Saúde Única

ABSTRACT

Homeless people are highly vulnerable in social, economic and health terms. Their living conditions cause not only high mortality but also higher morbidity from various diseases and illnesses compared to the general population. As a result, this study has raised concerns and questions about exposure to pathogens of zoonotic origin for this population and their contacts. Three articles were developed to assess the prevalence and risk factors for zoonoses in this population: the article in Chapter 1, entitled ":High SARS-CoV-2 seroprevalence in persons experiencing homelessness and shelter workers from a day-shelter in São Paulo, Brazil" investigated the prevalence and risk factors for SARS-CoV-2 in homeless people who attend a community center and employees of this place. A collection of biological material and an epidemiological questionnaire were carried out at a community center in August 2020 in the city of São Paulo. These collected samples were sent to the laboratory and processed, performing RT-PCR and ELISA tests for SARS-CoV-2. All swab samples were negative by RT-qPCR. IgG seropositivity was 111/203 (54.7%) in homeless people, 41/87 (47.1%) in employees, respectively. The risk factors were "age over 30" for homeless people and "self-declaration as non-white" and "loss of smell and taste" for employees. The variable "having a dog as a companion animal" was a protective factor for homeless people. Chapter 2 also used the same samples collected in 2020, but to assess the seroprevalence of Toxocara spp. in the same population. This resulted in a prevalence of 89/194 (45.9%,) in homeless people and 22/79 (27.8%, 95% CI: 19.2-38.6) in employees. Having a university degree was the only protective factor for Toxocara spp. infection. The third and final chapter presents the article: "One health approach on serosurvey of anti-Leptospira spp. in homeless persons and their dogs in South Brazil", which, unlike the previous ones, evaluated samples collected in three cities (São Paulo, Curitiba and Foz do Iguaçu). A total of 200 human and 75 dog samples were tested for anti-Leptospira spp. antibodies. No human samples were positive, while 5 (6.7%) of the dogs were positive.The three studies also looked at the sociodemographic profile of the population evaluated, which showed similar data to other studies that also evaluate this population, showing that most of these people are men aged between 20 and 50, self-declare themselves black or brown and have low levels of education. Therefore, the set of articles that make up this study shows that the homeless people and shelter workers evaluated here are more exposed to COVID-19 transmission than the general population. These two populations also had contact with Toxocara spp., indicating a relationship with their socio-environmental vulnerability. Living with dogs, on the other hand, was not a risk factor for leptospirosis transmission and the negative result in all the samples can supposedly be explained by a behavioral characteristic of constant

migration, thus avoiding contact with floods. This thesis points to the vulnerability of homeless people to exposure to pathogens of zoonotic origin, showing the importance and need for greater attention to be paid to this population.

Translated with DeepL.com (free version)Keywords; Vulnerability; SARS-COV2; Toxocariasis; Leptospirosis, One Health

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LISTA DE ABREVIATURAS

ACE2	Receptor da enzima conversora de angiotensina (do inglês Angiotensin-converting enzyme2)
AI	Índice de avidez
AT1	Receptor de Angotensina tipo 1
AWE	Extrato de Ascaris suum adulto (do inglês ascars suum adult worm extract)
CadÚnico	Cadastro Único
CAPS	Centro de assistência psicossocial
CI	Intervalo de confiança
COVID-19	Doença do coronavírus (do inglês corona virus disease 2019)
DDS	Determinantes sociais de saúde
DNA	Ácido desoxirribonucleico. Do inglês
ECA2	Enzima conversora de angiotensina
ELISA	Ensaio de imunoabsorção enzimática (do inglês enzyme-linked immunosorbent assay)
EPIs	Equipamentos de proteção individual
EUA	Estados Unidos da América
HCL	Ácido Clorídrico
HIV	Vírus da imunodeficiência humana
IBGE	Instituto Brasileiro de Geografia e Estatística
ICB	Instituto de ciências biológicas
IgG	Imunoglobulina G
IgM	Imunoglobulina M
IPEA	Instituto de pesquisa económica aplicada
U. K	Reino Unido. Do inglês United Kingdom
LPS	Lipolissacarídio (do inglês Lord Privy Seal)
LVCM	Laboratório de virologia clínica e molecular
MAT	Teste de aglutinação microscópica
NaOH	Hidróxido de sódio

OD	Densidade óptica
OR	Razão de chances (do inglês odds ratio)
PBST	Solução salina tamponada de trifosfato (do inglês Phosphate Buffered Saline whith Tween)
PCR	Reação em cadeia da polimerase
PSR	Pessoa em situação de rua
RBD	Receptor-Binding Domain
RI	Reactivity Index
RNA	Ácido ribonucleico
ROC	Característica de operação do receptor
RT qPCR	Reação em cadeia da polimerase em tempo real associada à transcrição reversa
SARS-Cov-2	Coronavírus 2 da síndrome respiratória aguda grave
SPP	Espécies
TB	Tuberculose
USP	Universidade de São Paulo
WHO	Organização mundial da saúde (do inglês Word Health Organization)

SUMÁRIO

1. INTRODUÇÃO

A população em situação de rua[1] está entre as populações mais vulneráveis no mundo, juntamente com os refugiados e pessoas privadas de liberdade (ALDRIDGE *et al*., 2018). Devido a fragilidade de sua situação socioeconômica e a falta da moradia, essas pessoas estão expostas a diversos agravos que trazem prejuízos a sua saúde (PINTO *et al*., 2014; CACCAMO *et al*., 2017; LUCHENSKI *et al*., 2018).

Desde 2012, a população em situação de rua no Brasil cresceu cerca de 140%, atingindo quase 222.000 pessoas em 2019, com mais da metade (56,2%) vivendo no sudeste do Brasil, principalmente na cidade de São Paulo (IPA, 2020).

Com os impactos da pandemia de COVID-19 foram adotadas diversas medidas para controle e prevenção da transmissão do vírus, como o isolamento social, uso de máscaras e frequente higiene das mãos (ZHENG *et al*., 2020). Porém, quando falamos de pessoas que não possuem um domicílio, nem tem acesso fácil a água e produtos de higiene e que dependem de doações para se alimentar, essas medidas tornam-se difíceis (OMEROV, 2019; THE LANCET, 2020). Mesmo após tantos registros de número de casos e de óbitos de COVID-19 na população geral, não se teve um número de pessoas em situação de rua afetadas pela pandemia. A falta de dados sobre o número de casos dificulta a criação de medidas efetivas para impedir que essas pessoas adoeçam e transmitam de forma arbitrária o vírus para outros indivíduos próximos (WENDEL *et al*., 2020); (ROEDERER *et al*., 2021).

Outra característica dessa população é o vínculo próximo com animais de companhia, principalmente os cães. A troca de afeto e cuidado pode ser muito benéfica para ambos (SCANLON *et al*., 2021), porém, com a fragilidade na condição de saúde dessas pessoas, é importante avaliar a presença de patógenos que tem em seu ciclo de vida cães e pessoas como hospedeiro.

[1] A partir desse ponto o termo “pessoas em situação de rua” virá abreviado como “PSR”

Dois exemplos de doenças zoonóticas que podem afetar tanto cães como humanos são a Leptospirose e a Toxocaríase. Ambas são doenças tropicais negligenciadas que atingem populações vulneráveis (ROSTAMI *et al.*, 2019). A proximidade frequente com cães pode fazer com que pessoas entrem em contato direto com fezes ou urina desses animais, bem como outros fatores consequentes da vulnerabilidade, tal como a falta de instrução, acesso precário a itens e condições de higiene e a impossibilidade de repousar em um ambiente salubre, podem acabar expondo ainda mais as pessoas em situação de rua à excretas de animais, do que o restante da população. (NEVES-SILVA; MARTINS; HELLER, 2018). O uso de ferramentas da biologia molecular como o RT qPCR e da imunologia ELISA e MAT, ajudam no diagnóstico das zoonoses e, usando-as de forma combinada, podem revelar o momento da infecção que aquele individuo se encontra e também auxiliam no apontamento da relação estatística de possíveis fatores de risco para a exposição a esses agentes. Com isso há a obtenção de dados sobre o comportamento epidemiológico desses patógenos nessa população.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a prevalência e possíveis fatores de risco das infecções por Covid-19 e toxicaríase em pessoas em situação de rua e funcionários de um centro de acolhimento de São Paulo e a prevalência de leptospirose em pessoas em situação de rua e seus cães.

2.2 OBJETIVOS ESPECÍFICOS

Realizar coletas de sangue e swabe nasal de PSR e funcionários de um Centro de acolhimento da cidade de São Paulo, com a finalidade de realizar exame sorológico (ELISA) e molecular (RTqPCR) para diagnostico de Covid-19 e toxocaríase;

Realizar coleta de sangue de pessoas em situação de rua e seus cães em três cidades brasileiras com a finalidade de realizar exame sorológico (MAT) para o diagnóstico de leptospirose;

Coletar informações por meio de questionário epidemiológico para realizar análise estatística e identificar possíveis fatores de risco do contacto com esses

patógenos correlacionando os resultados dos exames laboratoriais e as respostas dos questionários.

3. REVISÃO DE LITERATURA

3.1 POPULAÇÃO EM SITUAÇÃO DE RUA

3.1.1 Perfil demográfico e socioeconômico

A população em situação de rua à primeira vista é caracterizada como aquela que permanece rotineiramente nas ruas e que possui uma relação de dependência e convivência com os elementos presentes nos espaços públicos (SILVA *et al*., 2006). A circunstância de vida sem domicílio envolve uma variedade de contextos, a depender principalmente de fatores sociais, como a pobreza e exclusão social que caracteriza sua vulnerabilidade (YAZBEK *et al*., 2012). Estima-se que aproximadamente 150 milhões de pessoas estejam sem domicílio no mundo (C.D. H. N.U., 2020)

No Brasil, os dados sobre a população em situação de rua são limitados, uma vez que o censo demográfico do IBGE não considera as pessoas não domiciliadas como parte da população de pesquisa. Segundo o IPEA isso ocorre pela complexidade operacional de uma pesquisa de campo com pessoas sem endereço fixo, o que favorece a invisibilidade social da população (IPEA, 2015). Os dados levantados até o momento mostram que 300.868 pessoas vivem em situação de rua no Brasil e entre dezembro de 2012 e dezembro de 2023, o número de pessoas que vivem nas ruas da capital São Paulo aumentou 16,8 vezes, passando de 3.842 para 64.818 (SMADS, 2023). Já no Paraná, segundo registros do CadÚnico existem 13.384 PSR cadastradas. Desse total, cerca de 3.477 estão em Curitiba (SASPR, 2022).

O perfil social dessa população foi descrito em um censo realizado em São Paulo pela Secretaria Municipal de Assistência e Desenvolvimento Social que apontou que 85,5% são homens; 48,9% se declaram pardos; 26,1% brancos; 9,7% pretos; 2,4% indígenas e 1,1% amarelos. Já 51% das PSR da cidade têm entre 31 e 49 anos e a maior parte (23,9%) dessas pessoas não concluíram o ensino fundamental. Em relação ao estado de origem e o tempo de permanência nas ruas a maioria (55%) é oriundo do estado de São Paulo, seguido dos estados do Nordeste

(25,1%) e 52,5% declaram a perda de moradia a menos de três anos, enquanto 17,9% há mais de dez anos (SMADS, 2019).

3.1.2 Perfil sanitário

A saúde de um indivíduo depende de vários fatores, tanto individuas como coletivos e o conjunto desses fatores vão determinar uma condição sanitária favorável ou não. Quando falamos dos fatores coletivos, esses vão depender das condições em que as pessoas nascem, vivem, crescem, trabalham e envelhecem. Este conjunto de fatores é chamado de determinantes sociais da saúde (DSS), que engloba condições econômicas, políticas, culturais e ambientais (OMS, 2011). O próprio sistema único de saúde brasileiro coloca esse grupo como condicionantes de saúde, desta forma a saúde de uma população reflete a organização social e econômica do país (BRASIL,1990). Entre esses fatores, a moradia aparece como um determinante significativo da saúde (FITZPATRICK *et al*., 2013 ; GIBSON *et al*., 2011).

Tanto a moradia precária como a falta de acesso a moradia prejudicam direta e indiretamente a saúde das populações. Podemos citar questões como a insegurança alimentar, por vez que sua oferta precária, a dependência do fornecimento de terceiros através de doações ou mesmo a impossibilidade de armazenar e preparar o alimento de forma adequada tem efeitos diversos nas condições de saúde (PANIGASSI *et al*. 2008). Há relatos de PSR que procuram alimento no lixo (MONDRAGÓN-SÁNCHEZ *et al*., 2022), e o senso realizado em São Paulo constatou que 35,3% dos entrevistados relatam que passaram um dia inteiro sem comer nada (SMADS 2019). Esses relatos colaboram com o fato de que diversas doenças físicas são comummente associadas à falta de moradia, tais como: a desnutrição, dor crônica, doenças de pele e distúrbios musculoesqueléticos (HWANG, 2001)

Outra condição que está diretamente ligada com a falta da moradia é a qualidade do sono. Este é um aspecto que influencia no processo saúde/doença dessas pessoas e de forma diretamente proporcional, ou seja, com menos horas de sono por dia, pior a qualidade de saúde (THULIEN *et al*..., 2018). Essa condição precária de sono é reflexo de diversos fatores, principalmente o aspecto de

insegurança vivido nas ruas. Há uma necessidade de manter-se acordado durante a noite por questões de segurança, medo de sofrerem agressões, algo recorrentemente relatado (BARATA *et.al.*, 2015).

Essas condições descritas são alguns dos exemplos que prejudicam a saúde da população em situação de rua. Prejuízo que aumenta a morbidade e mortalidade das pessoas (NIELSEN *et al.*, 2011). Um estudo realizado na Finlândia mostrou que a falta de moradia está associada a um aumento de cinco vezes na mortalidade dessa população e que passam parte muito maior de suas vidas com saúde precária (STENIUS-AYOADE*et al.*, 2017). Já no Reino Unido, a expectativa de vida de uma pessoa em situação de rua é de 47 anos, em comparação com 77 anos para a população em geral. (TOMÁS, 2012).

Há poucos registros específicos da causa de mortalidade em PSR. Um trabalho realizado nos EUA identificou seis principais causas de morte nessa população, entre elas; overdose por drogas, doenças cardiovasculares, câncer, transtorno por uso de substâncias psicoativas, doença hepática e doença respiratórias (DICKINS *et al.*, 2023).

Comparando com a população geral, PSR tem uma morbidade significativamente maior para doenças infecciosas, como tuberculose, AIDS (síndrome da imunodeficiência adquirida) e Sífilis, assim como infecção por vírus da hepatite C e HIV (vírus da imunodeficiência humana) (FAZEI *et al.*, 2014), doenças respiratórias, como pneumonia, asma e doença pulmonar obstrutiva crônica (LEWER *et al.*, 2019), doenças de pele, sanguíneas, digestivas, ferimentos e envenenamento, além de uma precária_saúde bucal (FIGUEIREDO *et al.*, 2013; BENJAMINSEN *et al.*, 2020).

As doenças infecciosas aqui citadas são um grande problema para saúde pública e na população em situação de rua esse problema se agrava ainda mais, uma vez que prevalência do HIV 6,5 vezes maior do que a estimada para a população brasileira (PINHEIRO *et al.*, 2021). A Sífilis e a tuberculose também tem maior prevalência nessa população (SILVA *et al.*, 2014), sendo que o tempo médio de situação de rua está relacionado a uma maior frequência de tuberculose e infecção latente por tuberculose (GIOSEFFI *et al.*, 2022).

Há dois motivos principais para um quadro de tuberculose persistente que é a coinfecção por HIV, falha no tratamento da tuberculose, tendo em vista que no Brasil a população em situação de rua tem o dobro de risco de um tratamento malsucedido (RODRIGUES *et al*., 2023). Um estudo mostrou que a probabilidade de sucesso do tratamento da tuberculose cai em aproximadamente 50% na população em situação de rua brasileira. Além disso, a taxa de abandono do tratamento foi 2,9 vezes maior na população em situação de rua do que na população em geral, e a taxa de mortalidade por tuberculose foi 2,5 vezes maior na primeira (SANTOS e tal., 2021).

Um dos motivos que podem dificultar a adesão ao tratamento de diversas enfermidades são os transtornos e doenças mentais, que também estão associadas ao aumento da mortalidade, risco alto de doenças infecciosas (VÖLLM *et al...*, 2008) e a um aumento da exposição a violência (WALSH *et al*.,2003). Estudos realizados durante 20 anos encontraram taxas de prevalência ao longo da vida para doença mental entre 60% e 93,3% entre a população em situação de rua (FAZEL *et al*.,2008). Entre elas dependência de álcool, dependência de drogas ilícitas, transtornos de ansiedade, transtornos afetivos, depressão, doenças psicóticas, comprometimento cognitivo e transtornos de personalidade (FAZEL *et al*., 2008; SCHREITER *et al*., 2017).

Outra questão que também pode influenciar o insucesso de um tratamento é a dificuldade no acesso aos serviços de saúde (SANTOS *et al*., 2021). A falta de documentos de identidade e comprovante de residência dificulta o cadastro e o atendimento em alguns setores. Por se tratar de uma população sem domicílio, também não há local para guardar pertences pessoais, o que resulta na perda e roubo de seus pertences, por vezes retirados pelos próprios agentes de limpeza urbana, zeladoria e segurança pública (MONDRAGÓN-SÁNCHEZ *et al*., 2022). Essa dificuldade em armazenar itens pessoais impede não só o cadastro em sistemas públicos, mas também em manter medicamentos de uso prolongado, receitas médicas, pedido de exame e consulta, sem falar na dificuldade de entrar em contato com essa população (CONALOGUE *et al*., 2011).

Algumas vezes o atendimento é negado por falta de documentação e também há constantes relatos de constrangimento em estar em locais públicos e a

percepção de um tratamento menos acolhedor em virtude de suas vestimentas, odor corporal e modo de se portar (MONDRAGÓN-SÁNCHEZ *et al.*, 2022). Todos esses fatores contribuem para resistência ao acompanhamento médico, assim como as ações de prevenção de doenças.

No Brasil existe o Consultório na Rua, que foi instituído pela Política Nacional de Atenção Básica no ano de 2011. São equipes multiprofissionais que desenvolvem ações integrais de saúde frente às necessidades da população em situação de rua. Composta por 1.500 trabalhadores, entre enfermeiros, psicólogos, assistentes sociais, terapeutas ocupacionais, médicos, dentistas, profissionais de educação física e de arte e educação, entre outros (MACHADO *et al.*, 2021). Mas é importante lembrar que esse serviço é voltado para atenção básica, ou seja, não elimina a necessidade da utilização de outros setores e que o número de profissionais precisa atender um número expressivo de pessoas. (BRASIL, 2011).

A condição de vida dessa população também a expõe ao risco de entrar em contato com patógenos zoonóticos, já que ambientes superlotados e higiene precária aumentam a prevalência de infestação ectoparasitária e outros vetores (BROUQUI *et al.*, 2006). A higiene pessoal é uma das atividades de difícil execução pela população em situação de rua, sem um local adequado para tomar banho, se trocar e se limpar, além de estarem diretamente em contato com sujidades presentes nas ruas (MONDRAGÓN-SÁNCHEZ *et al*, 2022). Um estudo realizado em Boston, Massachusetts, entrevistou 194 PSR e 29% delas relataram ver ratos diariamente e 9,3% viram fezes de roedores. Este estudo constatou também que dormir na rua aumentava 13 vezes a chances visualizar roedores próximos ao local de descanso (LEIBLER *et al.*, 2018).

O contato com ectoparasitas como pulgas e piolhos podem transmitir doenças zoonóticas e anticorpos e DNAs para esses patógenos têm sido detectados frequentemente em PSR. *Bartonella quintana*, *Borrelia recurrentis* e Rickettsia prowazekii, são os agentes transmitidos por piolho mais comumente relatados nessa população (DENG *et al.*, 2023). No Brasil 109 indivíduos dessa população foram testados para Bartonella spp. E rickettsias do grupo tifo apresentando uma soroprevalência de 72,5% e 36,7% respectivamente (MARTÍNEZ *et al.*, 2023). Outra preocupação é que indivíduos HIV-positivos, tem um risco maior de um prognostico

pior para essas doenças e como já relatado aqui, há um número considerável de PSR com HIV+(LEIBLER *et al*., 2016).

3.1.3 Situação durante pandemia de CoviD-19

Em 2019 surgiu o novo coronavírus, que atingiu o mundo todo, tornando-se assim pandêmico. Essa pandemia afetou de forma mais drástica populações vulneráveis, devido às desigualdades socioeconômicas pré-existentes, e impactou o bem-estar social (LANCET, 2020).

Conforme novas descobertas sobre o vírus foram surgindo, medidas de contenção foram recomendadas, como uso de máscara, higiene de mãos e distanciamento social (TSAI *et al*., 2020).

Reconhecendo a vulnerabilidade dessa população diante da crise sanitária que se instalou, os órgãos municipais adotaram medidas especificas para prevenção da doença nessa população As principais medidas adotadas foram: abrigamento, por meio de lugares provisórios ou unidades para os doentes pela COVID-19; alimentação, por meio de restaurantes populares que ficarão abertos até nos fins de semana; orientação com os cuidados de saúde e oficinas; higiene, mediante à entrega de kits de higiene; saúde, por meio de vacinas contra gripe, triagem médica e consultório na rua (BRASIL, 2020). Ainda assim a recomendação de isolamento social permanece de difícil alcance, pois dependem de serviços para alimentação e pernoite, em sua maioria, em espaços coletivos. O uso de centros de acolhimento, permaneceu necessário (BRUNO *et al.*, 2023).

No Brasil não foram publicados dados oficiais sobre prevalência e mortalidade de PSR para SARS-Cov2, pela dificuldade de identificação dessa população e por não haver um registro próprio para elas. Em outros países do mundo houve relato de prevalência de PSR variando de 2,2% até 50% (APRIL *et al*., 2020; LOUBIERE *et al*., 2021). Essa diferença drástica deve-se ao período da pandemia que ocorreram as coletas, a situação de lotação dos abrigos e o tipo de teste realizado; para detecção de antígeno ou anticorpo. Por exemplo a prevalência de 2,2% foi encontrada em pessoas que dormiam nas ruas e no mesmo trabalho, 8,1% em pessoas que frequentavam abrigos de emergência (LOUBIERE *et al*., 2021). Fatores como compartilhamento de quarto com muitas pessoas, dormir em espaço comum, foi associado a uma maior probabilidade de infeção (GHINAI *et*

al.,2020; ROGERS *et al*., 2020) enquanto um aumento no número de banheiros privativos e uso de hotel com quarto individual para algumas pessoas foi associada a uma redução na infeção por *SARS-CoV-2*foi associado a uma menor prevalência (ROGERS *et al*., 2020; HUGGETT *et al*., 2021).

Mesmo com estratégias intensivas de controle de infecção (incorporando triagem diária de sintomas, testes de PCR frequentes e uso universal de máscaras) abrigos com grande densidade populacional tiveram alta incidência de COVID-19, indicando que medidas provavelmente não previnem surtos(CHAPMAN *et al*., 2021).

Além da população em situação de rua, alguns trabalhos avaliaram a prevalência de COVID-19 em funcionários dos abrigo, entendendo que essas pessoas então em contado direto com essa população, com isso se tornam contactantes e importantes para entender o ciclo epidemiológico da doença, tanto que essas duas populações tiveram prevalências semelhantes (ERIKSEN *et al*., 2022; APRIL *et al*., 2020).

3.2. *SARS-COV-2*

3.2.1 Classificação morfológica

O agente infeccioso da Covid-19 pertencente ao gênero Betacoronavirus, subfamília Coronavirinae, família Coronaviridae, ordem Nidovirales, reino Riboviria. e é um vírus classificado como genoma de RNA fita simples de cadeia positiva (GORBALENYA et al., 2020). Possui envelope, o nucleocapsídeo é formado por uma bicamada lipídica, na qual estão ancoradas as proteínas de espícula (S), membrana (M) e envelope (E). (HELMY et al., 2020; LI et al., 2020). Os trímeros de proteína S dos coronavírus formam espículas na superfície do vírus, dando-lhes aparência de coroa, o que deu origem ao nome “coronavírus” (HELMY et al., 2020). A proteína S é dividida em duas regiões: S1 (subunidade de ligação ao receptor); e S2 (subunidade de fusão de membranas). A região S1 compreende os domínios de ligação ao receptor, (RBD), que coordena a ligação do vírus ao receptor na célula hospedeira e são essenciais para determinar o tropismo celular e a capacidade de transmissão do vírus. (HEALD-SARGENT; GALLAGHER, 2012; LU et al., 2020).

3.2.2 Patogenia

O *SARS-CoV-2* infecta seus hospedeiros por meio da ligação da proteína S com o receptor da enzima de conversão da angiotensina 2 (ECA2), que é expresso em células endoteliais e em diversos órgãos, como: pulmões, coração, rins, testículos, fígado e intestino (LUAN *et al*., 2020). Inclusive pacientes com diabéticos e hipertensão possuem maior risco de desenvolver COVID-19 severa (GUAN; ZHONG, 2020); justamente pois possuem maior expressão de ACE2 causada por essas comorbidades e/ou pelo tratamento com inibidores de ACE (FANG, KARAKIULAKIS, ROTH, 2020).

A proteína S "pré-ativada" possui uma maior capacidade de mediar a fusão célula-célula, favorecendo a disseminação viral, já que, através desse mecanismo, os vírus podem evadir à ação do sistema imune do hospedeiro. (HOFFMANN; KLEINE-WEBER, PÖHLMANN, 2020). E sendo o padrão e a intensidade da resposta imune inata dos pacientes infectados pelo vírus um dos principais determinantes prognósticos da doença. (MATRICARDI, DAL NEGRO. NISINI, 2020).

Com a passagem do vírus na célula, o genoma do RNA é liberado no citoplasma, sendo o genoma traduzido por duas poliproteínas estruturais, o que inicia o processo de replicação viral (LI *et al...*, 2020).

O ACE2 é mais expresso principalmente nos pneumócitos tipo II, mas também tem expressão mais discreta em tecidos extrapulmonares como células do coração, rins, vasos sanguíneos e trato gastro-intestinal (YAN *et al.* 2020), e apresenta função fisiológica de degradar angiotensina II (ZOU *et al*. 2020). Quando há redução dos seus níveis, a concentração da angiotensina II, aumentando a ação do seu receptor (AT1), o aumento da sua ação provoca diversas reações celulares, como a, a vasoconstrição. Com isso ocorre o desequilíbrio do sistema renina-angiotensina afetando a função pulmonar, com a formação de edema, assim como, dano ao endotélio capilar (LI *et al*. 2020; KUBA *et al* 2020; IMAI *et al*. 2020)

Com morte das células pulmonares, o que provoca uma resposta imune local associada ao recrutamento de macrófagos, monócitos e linfócitos. O *SARS-CoV-2* é classificado como um vírus citopático, uma vez que o desencadeamento da destruição celular faz parte do seu ciclo de replicação no organismo do hospedeiro (TAY *et. al.,* 2020).

A coinfecção com outros vírus pode ocasionar um maio tempo de permanência viral no organismo, sendo a coinfecção com *HIV/SARS-CoV-2* preocupante para a gravidade do desenvolvimento da COVID-19 grave e morrer (KANWUGU, ADADI, 2021).

3.2.3 Características epidemiológicas

A emergência do *SARS-CoV-2* foi associada a mutações surgidas em um coronavírus de morcego, o animal reservatório natural da maioria dos coronavírus. (CORMAN *et al.*.., 2018; LI *et al.*., 2020). As mutações acumuladas no genoma do SARS-CoV levaram a substituições de aminoácidos em sua proteína de superfície viral que resultaram no aumento da afinidade de ligação desta com o receptor ACE2 humano, consequentemente, o vírus adquiriu maior capacidade de infectar células humanas, tornando-se mais virulento e adaptado à transmissão pessoa-pessoa, sendo esse fenómeno um condicionante que tornou esse vírus uma emergência para saúde humana (WALLS et al., 2020).

SARS-CoV-2 é transmitido primariamente de uma pessoa infectada sintomática ou não, a outra, através de partículas virais contidas em gotículas oronasais, expelidas no ar ou diretamente em superfície (ARONS et al., 2020; CORMAN *et. al.*., 2018; GANDHI; YOKOE; HAVLIR, 2020). No ar as partículas contaminadas podem permanecer infectantes por algum tempo, dependendo de vários fatores como confinamento do ambiente, umidade relativa do ar e carga viral, ou se permanecerem superfícies. No caso de partículas virais suspensas, a contaminação se dá pela inalação e contato com os olhos e a boca (VAN DOREMALEN et al., 2020)

A livre circulação do *SARS-CoV-2* em populações não imunizadas previamente proporcionou um crescimento do número de casos de modo exponencial. Com isso a necessidade da implementação de intervenções não-farmacológicas (INFs) como proposta de ações preventivas e de contenção, como: lavagem das mãos, distanciamento social, quarentena, uso de máscara, realização de testes em massa e vacinação (GARCIA, DUARTE, 2020).

No Brasil até mês de março de 2023 somava-se um total de 37.085.520 casos confirmados e 699.310 óbitos (BRASIIL, 2023).

3.3 *LEPTOSPIRA SPP.*

3.3.1 Classificação morfológica

A leptospirose que é causada por uma bactéria gram-negativa pertencente ao grupo das espiroquetas, é da ordem Spirochaetales, família Leptospiraceae, gênero *Leptospira* e têm espécies saprófitas e patogênicas e possuem membrana dupla e lipolissacarídeo (LPS) (FAINE *et al.,* 1999; LEVETT *et. al.,* 2010). O gênero *Leptospira spp*. possui 24 sorogrupos com mais 250 sorovares (PALANIAPPAN e al., 2006; CERQUEIRA, PICARDEU, 2009; ADLER, PEÑA MOCTEZUMA, 2010; LEVETT *et al*..., 2010). A diversidade antigênica entre sorovares das leptospiras é baseada na estrutura do componente carboidrato do LPS. Sorovares do mesmo grupo infectam diferentes espécies. Essa variação antigênica interfere no diagnostico, imunização e até patogenia da doença (LEVETT *et al*., 2010).

3.3.2 Patogenia

As leptospiras penetram no hospedeiro através da mucosa e da pele não íntegras. Por sua capacidade de quimiotaxia conseguem passar pelas membranas chegando ao sistema vascular, resultando em bacteremia. A leptospiras têm genes que codificam proteases e outros produtos que podem causar degradação da membrana celular do hospedeiro (AMAMURA *et al*., 2017). Além disso a leptospiras se ligam às células endoteliais que revestem os vasos sanguíneos e à VE-caderina vascular que é uma glicoproteina das junções de adesão endotelial, que mantém a integridade vascular. Essa ligação pode ser entendida como um dos principais fatores que contribuem para as manifestações hemorrágicas da doença (DE BRITO *et al*., 2018) Ela se multiplica em órgãos, mais comummente no sistema nervoso central, nos rins e no fígado. Posterior a lesão no endotélio dos vasos sanguíneos que evolui para uma isquemia localizada nos órgãos, isso leva uma necrose tubular renal, dano hepatocelular e pulmonar, meningite, podendo apresentar um quadro de icterícia, deficiência de plaquetas e consequente hemorragia generalizada (ADLER *et al*., 2010; MOHHAMED, 2011).

Outro mecanismo da bactéria é poder passar entre as células epiteliais, incluindo as que revestem os túbulos renais, podendo assim colonizar dos túbulos proximais,

onde são excretadas na urina por semanas ou meses (ADLER *et al*., 2010; MOHHAMED, 2011).

3.3.3 Características epidemiológicas

A transmissão da bactéria ocorre de forma direta por contato com a urina de animais infectados, como roedores, animais silvestres e cães, ou indireta, atrávez da agua contaminada com a urina dos mesmos. O homem torna-se hospedeiro acidental dessa bactéria principalmente quando entra em contato esgoto a céu aberto, resíduos de enchente ou diretamente com urina do animal infectado (ZUBACH *et al*., 2015)

Apesar do roedor ser o principal reservatório dessa bactéria, o cão tem importante papel epidemiológico para manutenção dela do meio ambiente. (CASANOVAS-MASSANA *et al*., 2021). Fatores socioambientais, incluindo infraestruturas e saneamento inadequados, contaminação da água e do solo, acúmulo de lixo e proliferação de roedores contribuem para disseminação da doença (FELZEMBURGH *et al*., 2014; HAGAN *et al*., 2016).

Apesar de ser uma doença de distribuição mundial é considerada negligenciada com subnotificação de casos. No Brasil é endêmica e tem picos epidêmicos em épocas de fortes chuvas, no período de 2009 a 2019, foram confirmados 41.602 casos de leptospirose humana no território nacional (GALAN *et al*.,2021). Muitas zoonoses têm como característica epidemiológica afetarem de forma mais recorrente populações vulneráveis, isso deve-se ao fato da exposição que essas pessoas têm ao patógeno e de sua pré-condição de saúde. Vivendo em áreas com precária condição ambiental, pouco acesso à educação e qualidade sanitária deficitária, essas pessoas passam a ter maior probabilidade de infecção por agentes nocivos (RONCARATI *et al*.,2018).

3.4 *TOXOCARA SPP. SPP.*

3.4.1 Classificação morfológica

O *Toxocara spp.* é um nematódeo que pertence à família ascaridae e as duas espécies principais de *Toxocara spp.* que afeta os humanos são o *Toxocara canis e Toxocara cati*. Seus hospedeiros definitivos são gatos, cães e alguns

canideos silvestres que abrigam os parasitas em seu intestino, eliminando ovos em suas fezes, podendo esses permanecer com potencial infeccioso por anos no ambiente (MA *et al*., 2018).

Os ovos pertencentes do nematódeo são subglobulares, de cápsula espessa e rugosa, já os adultos apresentam corpo cilíndrico, não segmentado, robusto, esbranquiçado e revestido por espessa camada quitinosa. Na extremidade anterior do parasito as expansões cuticulares possuem forma e tamanhos diferentes de acordo com a espécie, sendo ferramenta de identificação pela microscopia estereoscópica (TEYLOR *et al*., 2010)

3.4.2 Patogenia

Hospedeiros intermediários ingerem os cistos que eclodem e migram para diversos órgãos, após isso o *Toxocara spp.* permanece em seu intestino. O parasito migra através dos tecidos extra-intestinais (WINDERS *et al*., 2020). Após a migração da larva através dos tecidos por meio de um mecanismo de digestão de protease, ocorre resposta imune do hospedeiro e com isso um processo de inflamação local (SALEM,1992). Esse processo inflamatório ocorre nos olhos, no sistema nervoso central, principalmente em adultos, fígado e sistema respiratório, gerando hepatomegalia e asma em crianças (LI *et al.,* 2014; FINSTERER *et al.,* 2007; PIVETTI-PEZZI *et al*., 2009).

3.4.3 Características epidemiológicas

Os causadores dessa doença são principalmente por dois nematoides o *Toxocara canis* e *Toxocara cati*, que tem como seus hospedeiros o cão e o gato (FISHER *et al*., 2003). O ser humano entra como hospedeiro acidental desse patógeno, a convivência de cães e pessoas no mesmo lugar, ainda mais quando são pessoas que dormem em praças e parques, pode gerar esse ciclo de infecção, caso esses cães não tenham sido devidamente desverminados (NEGRI *et al*., 2013).

Com o aumento populacional de cães e gatos nos centros urbanos, como jardins, praças, parques e campos de areia e, muito deles de hábito errante, sem o devido controle parasitário, ocorre maior taxa de transmissão de zoonoses, como a toxocariase, (FISHER *et al*., 2013; MANINI *et al*., 2012). (FU *et al*., 2014; GIBBONS *et al*., 2001).

A transmissão para seres humanos ocorre pela via fecal-oral, quando acidentalmente ingere ovos de *Toxocara spp. presentes* no alimento, na água ou solo contaminado com fezes de cães (GIBBONS *et al.*, 2001). Entre os fatores de risco para o contato com o parasita estão pobreza, idade jovem, latitude, contato com solo contaminado e alta taxa e população canina e felina frequentando praças e parques (WINDERS *et al.*, 2022). A toxocariase também é uma doença tropical que pode estar vinculada com a falta de saneamento básico.

4. HIGH *SARS-CoV-2* SEROPREVALENCE IN PERSONS EXPERIENCING HOMELESSNESS AND SHELTER WORKERS FROM A DAY-SHELTER IN SÃO PAULO, BRAZIL

4.1 ABSTRACT

Brazil presents one of the highest COVID-19 death tolls in the world. The initial SARS-CoV-2 epicenter was São Paulo city. As of 2019, the homeless population of São Paulo city was estimated at 24,344 individuals, the largest national homeless population. The present study aimed to concomitantly assess the molecular and serological prevalence and associated risk factors of SARS-CoV-2 infection in a homeless population and related shelter workers from a day-shelter. Serum samples, nasopharyngeal and oropharyngeal swabs of persons who are homeless and shelter workers collected from August 25th to 27th, 2020 were tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies by ELISA and SARS-CoV-2 RNA by RT-qPCR, respectively. All swab samples tested negative by RT-qPCR. Seropositivity of IgM and IgG was 5/203 (2.5%) and 111/203 (54.7%) in persons who are homeless, and 5/87 (5.7%) and 41/87 (47.1%) in shelter workers, respectively, with no statistical differences between groups. The high seroprevalence found herein indicates early environmental and urban spreading of SARS-CoV-2, associated with sociodemographic and economic vulnerability.

4.2 BACKGROUND

The current *SARS-CoV-2* pandemic has severely affected Latin America, particularly Brazil, currently presenting one of the highest active transmission rates among 48 countries, with 16,720,081 confirmed cases and 467,706 deaths as of June 1st, 2021 [1]. Due to pre-existing socioeconomic inequalities, the novel coronavirus spread has affected vulnerable populations worldwide and impacted human social welfare [2]. The pandemics has also increased their vulnerability as a consequence of social and economic losses, associated with disparities in policy responses, particularly in emerging countries [3].

Brazil has been ranked as the largest and most unequal Latin American country in income distribution (Gini index of 0.540 in 2018) [4], with inequality rising since 2014 as a result of economic crisis and political turmoil, with 13.6 million people living in extreme poverty, 6.5% of the overall nationwide population [4]. Aggravated by the *SARS-CoV-2* pandemic, extreme poverty in Brazil has been expected to rise 9.5% by the end of 2020 [5], leading to an increase in homelessness, particularly in major urban centers. Since 2012, the homeless population in Brazil has grown around 140%, reaching almost 222,000 people in 2019, with more than half (56.2%) living in south-eastern Brazil, mainly in Sao Paulo city [6]. As the most populous Brazilian city and the fourth worldwide, São Paulo had an estimated homeless population around 24,000 persons in 2019. In addition to insufficient healthcare access, inadequate nutrition, and inability to prevent *SARS-CoV-2* transmission due to precarious living conditions [3], such population has presented multiple comorbidities, such as drug addiction, sexually transmitted and other infectious diseases, and non-communicable diseases, with some being associated with worsening the clinical onset of *SARS-CoV-2* infection [7,8].

Few studies have been conducted on *SARS-CoV-2* detection in persons experiencing homelessness or shelter workers, mostly taken at the beginning of local epidemics between March and April 2020. In the USA, the prevalence of *SARS-CoV-2* by RT-qPCR in cohabitants and support service workers (shelter workers) of various institutions varied from 48/533 (9.0%) in Seattle, and 162/458 (35.4%) and 147/408 (36.0%) in Boston, 105/206 (50.9%) in San Francisco, 11/308 (3.6%) in Atlanta, and 18/118 (15.2%) and 19/181 (10.5%) in King County, Washington State [9,10]. No survey to date has been conducted in Brazilian homeless populations. Thus, this study aimed to concomitantly assess the molecular and serological prevalence and associated risk factors of *SARS-CoV-2* infection in a homeless population and shelter workers from a day-shelter in São Paulo City, the urban epicenter of *SARS-CoV-2* transmission in Brazil at the time of the survey.

4.3 MATERIAL AND METHODS

4.3.1 Local of study

This is a cross-sectional study of a homeless population and related shelter workers. These shelter workers were healthcare and assistance professionals (e.g.,

nurses, social workers, administrative personnel, cooks, cleaning, and maintenance professionals) providing care to people who were homeless. The study was conducted in the city of São Paulo (23˚33'1"S, 46˚ 38'2"W), capital of São Paulo State, south-eastern Brazil, ranked as the second largest Gross Domestic Product (GDP) and the most populated city in Latin America, with 11,253,500 habitants, a high Human Development Index (HDI) (0.805), humid subtropical climate and average temperatures varying from 19˚C (winter) to 25˚C (summer) [11].

Sample collection was performed in the consecutive working days of August 25th, 26th, and 27th, 2020, at a major shelter for people who are homeless called the Community Center of São Martinho de Lima, located in the Mooca subregion, area with the second highest homeless population, with 4,779 individuals corresponding to 19.6% of the total homeless population (n = 24,344) of São Paulo City [12]

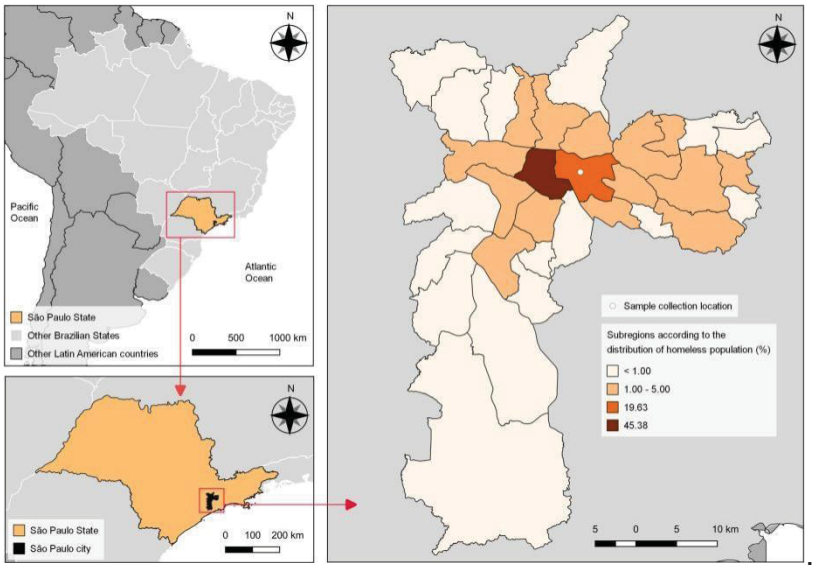

Fig 1. Geographical location of the shelter and distribution of homeless population as described in the latest São Paulo city survey [12].

The center is a day-only public service with no dormitory or sleepover, providing three daily meals and medical assistance to persons who are homeless. The shelter serves around 600 breakfast meals and 800 lunch meals daily. The exact number of persons experiencing homelessness accessing the center each day is unknown. In a scenario where 800 people access the shelter daily (i.e., the maximum capacity for lunch meals), we calculated a sample size of 204 people for this study (50% *SARS-*

CoV-2 prevalence,90% CI, and 5% error). In addition, the shelter has around 90 workers that assist with cleaning, cooking, and maintenance, in addition to social and healthcare professionals.

Individuals who are homeless were informed about the COVID-19 testing research by public announcement as they entered the shelter. The reason why these individuals were entering the shelter was not asked and they voluntarily entered the sampling line. As no individual invitation was made for participation, no calculation of refusal rate was possible. The interviewers and medical team were stationed inside the first and largest room of the center and worked every day from 7 am (before breakfast) to around 3 pm (after lunch). Meanwhile, shelter workers were informed about the COVID-19 testing research two days prior to sampling. They also voluntarily came to sampling lines and were received by the interviewers and medical team. All shelter workers from the center participated in the study. Participants were first informed about the study, provided signed consent, responded to the questionnaire, and then were subjected to blood and swab collection. To be included in the study, participants needed to have answered the questionnaire and collected both blood and swab samples.

This study has been approved by the Ethics Committee in Research at the Federal University of Parana´ (CAAE: 80099017.3.0000.0102, protocol number: 2.512.196), by the Municipal Ethics in Health Committee, São Paulo Secretary of Health (CAAE: 80099017.3.3004.0086, protocol number: 3.366.684) and by the Ethics Committee Research of the Clinical Hospital from the Federal University of Paraná (CAAE: 80099017.3.3005.0096, protocol number: 3.623.845), linked to the National Human Ethics Research Committee of the Brazilian Ministry of Health. The investigation was carried out in coordination with the shelter service providers, healthcare providers, and universities. All participants (who were all > 18 years old) were informed and signed a written consent.

4.3.2 Application of a structured epidemiological questionnaire

All participants (both persons who are homeless and shelter workers) were interviewed with a structured questionnaire for sociodemographic, behavioral, and clinical information. For the homeless population, questions evaluated sociodemographic aspects (city of origin, age, gender, self-identified race/ethnicity,

and level of education), previous assistance by the government's healthcare program "Street Clinics" ("Consultório na Rua"), previous assistance by counselling and psychological services (CAPS), current drug use (alcohol, tobacco, marijuana, cocaine, crack, injectables–yes or no questions), number of years without permanent housing, reasons for homelessness (unemployment, alcohol/drugs, familiar conflict, others), use of face mask, previous contact with someone positive for *SARS-CoV-2*, current or previous (last three months) *SARS-CoV-2* symptoms (fever, difficulty breathing, tiredness, body pain or discomfort, throat pain, diarrhea, chest pain, dry cough, loss of sense of smell or taste, headache), previous *SARS-CoV-2* testing, ownership of companion animal(s) (dog, cat, other), previous health history (HIV, syphilis, hepatitis, cardiovascular disease, tuberculosis, diabetes, other comorbidities), and access to water and soap to wash hands.

For shelter workers, questions evaluated sociodemographic aspects (city of origin, age, gender, self-identified race/ethnicity, level of education), current drug use (tobacco, marijuana, cocaine, crack, injectables–yes or no questions), use of face mask, previous contact with someone positive for *SARS-CoV-2*, current or previous (last three months) *SARS-CoV-2* symptoms (fever, difficulty breathing, tiredness, body pain or discomfort, throat pain, diarrhea, chest pain, dry cough, loss of sense of smell or taste, headache), previous *SARS-CoV-2* testing, ownership of companion animal (dog, cat, other), previous health history (HIV, syphilis, hepatitis, cardiovascular disease, tuberculosis, diabetes, other comorbidities), and access to water and soap to wash hands. All answers provided by the participants (individuals who are homeless or shelter workers), including symptoms and comorbidities, were self-reported.

4.3.3 Sample collection

Nasopharyngeal and oropharyngeal swabs and whole blood samples were collected from individuals by trained nurses and subjected to *SARS-CoV-2*-specific RT-qPCR and ELISA (IgM and IgG) assays, respectively. Briefly, nasopharyngeal and oropharyngeal swabs from each patient were jointly stored in a cryotube containing 1 mL of lysis buffer (NucliSENS easyMag, BioMerieux, Lyon, France) for virus inactivation and preservation. Blood samples and cryotubes containing the swabs were refrigerated and processed on the same day at the Institute of

Biomedical Sciences (ICB), University of São Paulo (USP), Brazil. The serum was separated after centrifugation of the whole blood samples at 2,500 x g for 10 minutes and stored at 4˚C until testing.

4.3.4 *SARS-CoV-2* RT-qPCR

Nasopharyngeal and oropharyngeal swabs were processed in the Laboratory of Clinical and Molecular Virology (LVCM), ICB, USP for molecular *SARS-CoV-2* testing by a specific RTqPCR [13]. First, total RNA was extracted from 400 µL of sample using NucliSENS easyMag fully automated platform (BioMerieux, Lyon, France). The RT-qPCR assay was then carried out using an adapted protocol developed at the Charite´ Institute of Virology, University of Berlin, Germany [13]. The positive control consisted of RNA extracted from Vero-E6 cell culture infected with *SARS-CoV-2* (SARS.COV-2/SP02/human2020/Br, GenBank accession number MT126808.1), and the negative control was ultrapure water. In addition, RT-qPCR for the housekeeping gene human RNase P (RNP) was run to ensure RNA integrity, sample quality, and absence of inhibitors, as described previously [14]. All samples were run in duplicates.

4.3.5 Serological Test

IgM and IgG antibodies against the *SARS-CoV-2* nucleocapsid (N) protein were measured using a previously developed ELISA assay [15]. Briefly, 96-well polystyrene microliter plates (Corning, NY, USA) were coated with 100 µL of Ncov-PS-Ag7 antigen (Fapon Biotech Inc, Dongguan, China) at a concentration of 0.2 µg/mL in 0.05 M sodium carbonate buffer (pH 9.6) for one hour at 37˚C. The plates were then washed with 1X phosphate-buffered saline with 0.05% Tween 20 (PBST) five times and blocked with 300 µL/well of blocking buffer (Advagen Biotech1, São Paulo, Brazil) for 3 hours at 37˚C. A total of 10 µL of each serum sample was diluted at 1:50 for IgM, and 1:100 for IgG in diluent solution added to each well and incubated for one hour at 37˚C. Following five washes with PBST, bound antibodies were detected using goat anti-human IgM (1:4,000) or IgG (1:4,000) conjugated with horseradish peroxidase (Sigma-Aldrich Co., Steinheim, Germany). Immunoglobulin detection was revealed after five washes with PBST and 10 minutes incubation with tetramethylbenzidine (Invitrogen, California, USA) at room temperature. After stopping the reaction with 0.2 N sulfuric acid, the optical density (O.D.) was

measured at 450 nm. Two positive serum samples and three negative serum samples were used as controls. The two positive serum samples were from symptomatic patients confirmed to be infected with *SARS-CoV-2* by RT-qPCR, while the negative serum samples were from the pre-pandemic period. For IgM, the cut-off value was determined using the average O.D. of the three negative serum samples plus three standard deviations, while for IgG was set as 0.4, as previously described [15].

4.3.6 Data collection and statistical analysis

To identify the pandemic's epidemiological moment when the survey was carried out, data regarding reported cases and deaths from March 28th (first case of *SARS-CoV-2* detected in Brazil) to November 19th, 2020 in São Paulo city were retrieved from the official records of the Brazilian Ministry of Health (https://susanalitico.saude.gov.br/extensions/covid-19_html/ covid-19_html.html) and plotted against time using Microsoft Excel 365. Questionnaire data and serological test results were organized in spreadsheets and analyzed in R software, version 4.0.3 [16] to verify associations between studied variables and serology results for SARS-CoV- 2 (IgG). The positive results between groups were compared with Pearson's chi-square test. For each group, a bivariate analysis for all independent variables was performed by calculating the Odds Ratio (OR), the Confidence Interval (CI) for OR and the p-value, with a confidence level (α) of 5%. Then, a multivariate analysis was performed fitting variables in a logistic regression model (stepwise logistic regression). Using a forward stepwise approach and adjusting the models for age and sex, the best fitting model was the one including significantly associated variables ($p < 0.05$) and minimizing the Akaike's Information Criterion (AIC) value.

Constant variables—those that all respondents gave the same answer to–or collinear variables were excluded from the final model, as well as variables with more than 10% of missing data. These include: for the homeless population–years experiencing homelessness (missing values = 52), reasons for homelessness (unemployment, alcohol/drugs, familiar conflict, others) (missing values = 29), companion animal (other) (Constant = no); and for shelter workers —drug use (cocaine, crack, injectables) (Constant = no), selected current or previous SARSCoV-2 symptoms (yes x no) (difficulty breathing, tiredness, throat pain, diarrhea, chest

pain, dry cough) (Collinearity), previous health history (yes x no) (hepatitis, tuberculosis) (Constant = no).

Health histories, including infectious diseases, diabetes, and cardiovascular diseases, were compared between persons who are homeless and shelter workers using Pearson's chi-square or Fisher exact tests. Optical density distributions of IgG titers were compared between populations using Mann-Whitney test. Results were considered significant when $p < 0.05$.

4.4. RESULTS

A total of 203 individuals who are homeless and 87 shelter workers participated in the study. Twenty-nine additional individuals experiencing homelessness answered only the questionnaire but refused to be sampled, and other four refused to collect swab samples; these were all excluded from the study. The 290 swab and serum samples were submitted to SARS-CoV- 2-specific RT-qPCR and ELISA testing. Successful amplification of housekeeping gene RNP control has indicated that all RNA samples were suitable for testing, with Ct values ranging from 23.1 to 29.9 (mean = 26.1, stdev = 1.4). None of the 290 RNA samples from swabs was positive in the *SARS-CoV-2*-specific RT-qPCR assay, indicating the absence of active infection in the surveyed populations.

In contrast, IgM and IgG antibodies were found in 5/203 (2.5%, CI 0.3–4.6%) and 111/203 (54.7% CI 47.8–61.5%) persons who are homeless, and in 5/87 (5.7%, CI 0.9–10.6%) and 41/87 (47.1%, CI 36.6–57.6%) shelter workers, with no statistical differences between the two populational groups (IgM $p = 0.291$; IgG $p = 0.251$). Presence of IgM in the absence of IgG was not observed in the individuals who are homeless, while two (2.3%) shelter workers had circulating IgM and no IgG. Optical densities for the 10 IgM-positive individuals ranged from 0.431 to 1.967, while the distributions of the optical density values of IgG obtained are shown in with significant difference in ELISA titer being detected between individuals who are homeless and shelter workers ($p = 0.025$).

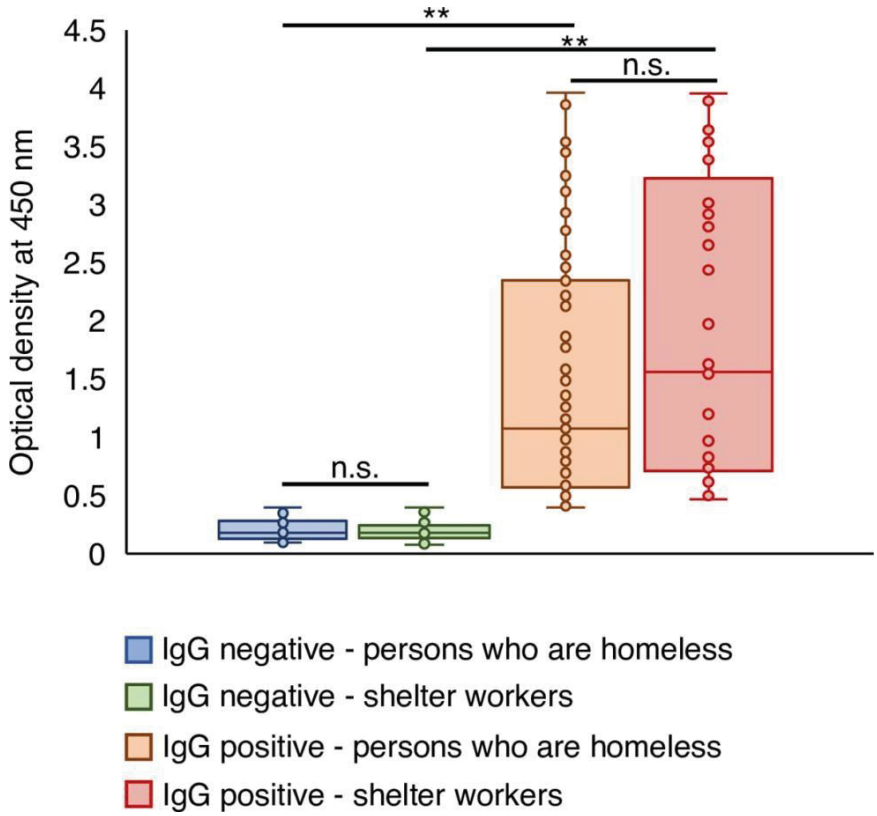

Fig 2. Optical density at 450 nm obtained for the ELISA assay used to detected anti-SARS-CoV-2 IgG in persons who are homeless (n = 203) and shelter workers (n = 87) attending the Community Center of São Martinho de Lima, Mooca subregion, São Paulo, Brazil.

These findings indicate that *SARS-CoV-2* infection in thesepopulations was not a recent event, corroborating with the negative results observed in the *SARS-CoV-2* RT-qPCR. As very few individuals had IgM antibodies only, IgG seropositivity was considered the gold standard to indicate previous *SARS-CoV-2* infection and was used in the risk factor analysis.

Using official data from the Brazilian Ministry of Health of reported new cases and deaths of *SARS-CoV-2* in São Paulo city, at the time of the survey, a decline in the numbers from its first initial peak (end of June 2020 for deaths) was observed, presenting a moving average from 1,706.86 to 1,768.86 new cases and from 59.29 to 64.86 deaths reported daily at that epidemiological week [17]

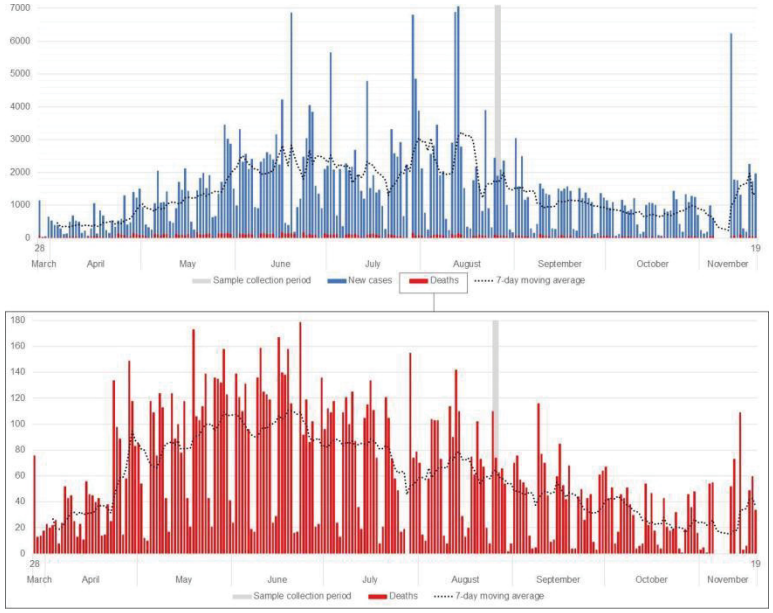

Figura 3.Number of SARS-CoV-2 new cases (upper graph, blue) and deaths (upper and bottom graphs, red) officially reported in São Paulo city, Brazil, from March 28th (first case of SARS-CoV-2 detected in Brazil) to November 19th, 2020, with a 7-day moving average. Days of sample collection are shown as grey bars (August 25th to 27th, 2020).

The containment phase was determined as orange (from a red to green scale, where green was medium risk with fewer restrictions, and red was very high risk with essential activities only) by São Paulo Health Authorities. Thus, at the time of the survey, the first highest peak of deaths by COVID-19 in the city had passed, although viral transmission was not controlled.

The descriptive statistics of the surveyed population is presented in Tables 1 and 2. Contrasting sociodemographic characteristics (except for age) and health histories were observed between homeless and shelter worker populations. While the majority of the shelter workers were born in São Paulo (61.2%), individuals who are homeless were mostly (68%) from other Brazilian cities, reflecting the well-described phenomena of migratory movements of vulnerable populations to large urban centers in the country [18]. Additionally, persons who are homeless were mostly male (89.1%), while the shelter worker population was well distributed between males and females. Furthermore, most individuals who are homeless identified themselves as Brown (mixed-race Brazilians) (42.9%), followed by white (29.5%), Black (26.1%), and Indigenous Brazilian (1.5%), while the shelter workers were somewhat equally

represented by white (35.3%), Black (31.8%), and Brown (32.9%). Finally, 89.7% of the shelter workers had high school and/or higher education diplomas compared with 52.2% of the homeless population.

When considering the health histories of both populations, the homeless population was significantly more affected by syphilis and tuberculosis when compared to shelter workers (p = 0.0009 and p = 0.0002, respectively). Previous or current history of HIV infection, syphilis, hepatitis, and tuberculosis were declared by 6.5%, 15.1%, 8.5%, and 11.4% of the respondent individuals experiencing homelessness, respectively (Table 1). Cardiovascular disease and diabetes were reported by 29.1% and 9.4% of
individuals who are homeless, respectively (Table 1).

TABLE 1 - DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF ASSOCIATED RISK FACTORS FOR SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 203 PERSONS WHO ARE HOMELESS OF SÃO PAULO, BRAZIL.

Variables		Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	N	%	n	%	n	%				
													(CONTINUA)
Sociodemographic aspects	**Origin**												
	São Paulo	30	32.6	35	31.5	65	32.0	203	100.0		Ref		
	Others	62	67.4	76	68.5	138	68.0			1.05	**0.58**	**1.90**	**0.870**
	Age *												
	Young (18–30 years-old)	21	22.8	8	7.3	29	14.4				Ref		
	Adult (31–60 years old)	62	67.4	79	71.8	141	69.8	202	99.5	3.35	**1.44**	**8.51**	**0.007**
	Elderly (> 60 years old)	9	9.8	23	20.9	32	15.8			6.71	**2.27**	**21.75**	**< 0.001**
	Gender												
	Male	82	90.1	98	88.3	180	89.1				Ref		
	Female	8	8.8	12	10.8	20	9.9	202	99.5	1.26	**0.50**	**3.34**	**0.636**
	Others	1	1.1	1	0.9	2	1.0			0.84	**0.03**	**21.38**	**0.900**
	Race/Ethnicity												
	White	30	32.6	30	27.0	60	29.6				Ref		
	Black	20	21.7	33	29.7	53	26.1			1.65	**0.78**	**3.50**	**0.191**
	Mixed (Brown—Pardo)	41	44.6	46	41.4	87	42.9			1.12	**0.58**	**2.17**	**0.732**
	Indigenous Brazilian	1	1.1	2	1.8	3	1.5	203	100.0	2.00	**0.17**	**23.25**	**0.580**
													(CONTINUAÇÃO)

	Level of education												
	Higher education	7	7.6	12	10.8	19	9.4				**Ref**		
	High school	46	50.0	41	36.9	87	42.9	203	100.0	0.52	**0.19**	**1.45**	**0.210**
	Elementary school	39	42.4	58	52.3	97	47.8			0.87	**0.31**	**2.40**	**0.784**
Previous assistance	**Assistance by the government healthcare program**												
	Yes	27	29.7	40	37.4	67	33.8	198	97.5		**Ref**		
	No	64	70.3	67	62.6	131	66.2			0.71	**0.39**	**1.28**	**0.254**
	Assistance by Psychosocial Care Centers												
	Yes	26	28.3	29	26.4	55	27.2	202	99.5		**Ref**		
	No	66	71.7	81	73.6	147	72.8			1.10	**0.59**	**2.05**	**0.763**
Behavior characteristics	**Alcohol**												
	No	34	37.0	46	41.4	80	39.4	203	100.0		**Ref**		
	Yes	58	63.0	65	58.6	123	60.6			0.83	**0.47**	**1.46**	**0.515**
													(CONTINUAÇÃO)
	Tobacco *												
	No	31	33.7	59	53.2	90	44.3	203	100.0		Ref		
	Yes	61	66.3	52	46.8	113	55.7			0.45	0.25	0.79	**0.006**
	Marijuana *												
	No	55	59.8	88	79.3	143	70.4	203	100.0		Ref		
	Yes	37	40.2	23	20.7	60	29.6			0.39	0.21	0.72	**0.003**
	Cocaine												

	No	55	59.8	80	72.1	135	66.5	203	100.0		Ref		
	Yes	37	40.2	31	27.9	68	33.5			0.58	0.32	1.04	0.066
	Crack												
	No	69	75.0	87	78.4	156	76.8	203	100.0		Ref		
	Yes	23	25.0	24	21.6	47	23.2			0.83	0.43	1.60	0.570
	Injectables												
	No	84	93.3	111	100.0	195	97.0	201	99.0		Ref		
	Yes	6	6.7	0	0.0	6	3.0			0.06	0.00	1.05	0.054
Years experiencing homelessness	**Years on the streets**												
	Less than one year	23	33.3	28	34.1	51	33.8	151	74.4		Ref		
	One year or more	46	66.7	54	65.9	100	66.2			0.96	0.49	1.90	0.916
Reasons for homelessness	**Unemployment**												

(CONTINUAÇÃO)

	No	38	48.1	42	44.2	80	46.0	174	85.7		Ref		
	Yes	41	51.9	53	55.8	94	54.0			1.17	0.64	2.13	0.608
	Alcohol/drugs												
	No	53	67.1	69	72.6	122	70.1	174	85.7		Ref		
	Yes	26	32.9	26	27.4	52	29.9			0.77	0.40	1.47	0.427
	Familiar conflict												
	No	47	59.5	53	55.8	100	57.5	174	85.7		Ref		
	Yes	32	40.5	42	44.2	74	42.5			1.16	0.64	2.14	0.623
	Other												
	No	76	95.0	92	96.8	168	96.0	175	86.2		Ref		
	Yes	4	5.0	3	3.2	7	4.0			0.62	0.12	2.89	0.539
Use of face mask during pandemic	**Face mask use**												
	Yes	75	83.3	97	88.2	172	86.0	200	98.5		Ref		

Contact history	No	15	16.7	13	11.8	28	14.0			0.67	0.30	1.49	0.328
	Contact with someone positive												
	No	68	75.6	80	73.4	148	74.4	199	98.0		Ref		
	Yes	22	24.4	29	26.6	51	25.6			1.12	0.59	2.14	0.728
Current or previous (last 3 months) COVID-19 symptoms	**Fever**												
												(CONTINUAÇÃO	
	No	74	80.4	80	72.1	154	75.9	203	100.0		Ref		
	Yes	18	19.6	31	27.9	49	24.1			1.59	0.83	3.13	0.168
	Difficulty breathing												
	No	67	72.8	76	68.5	143	70.4	203	100.0		Ref		
	Yes	25	27.2	35	31.5	60	29.6			1.23	0.67	2.29	0.499
	Tiredness												
	No	68	73.9	77	69.4	145	71.4	203	100.0		Ref		
	Yes	24	26.1	34	30.6	58	28.6			1.25	0.68	2.33	0.476
	Body pain												
	No	63	69.2	86	77.5	149	73.8	202	99.5		Ref		
	Yes	28	30.8	25	22.5	53	26.2			0.65	0.35	1.23	0.186
	Throat pain												
	No	74	80.4	86	77.5	160	78.8	203	100.0		Ref		
	Yes	18	19.6	25	22.5	43	21.2			1.20	0.61	2.39	0.608
	Diarrhea												
	No	77	83.7	97	87.4	174	85.7	203	100.0		Ref		
	Yes	15	16.3	14	12.6	29	14.3			0.74	0.33	1.63	0.455
	Chest pain												
	No	73	79.3	88	79.3	161	79.3	203	100.0		Ref		
	Yes	19	20.7	23	20.7	42	20.7			1.00	0.51	2.00	0.990

(CONTINUAÇÃO)

	Dry cough												
	No	63	68.5	86	77.5	149	73.4	203	100.0		Ref		
	Yes	29	31.5	25	22.5	54	26.6			0.63	0.34	1.18	0.150
	Loss of sense of smell or taste												
	No	77	83.7	90	81.1	167	82.3	203	100.0		Ref		
	Yes	15	16.3	21	18.9	36	17.7			1.20	0.58	2.52	0.628
	Headache												
	No	68	73.9	78	70.3	146	71.9	203	100.0		Ref		
	Yes	24	26.1	33	29.7	57	28.1			1.20	0.65	2.24	0.566
Previous COVID-19 test	**Previous COVID-19 test**												
	Yes	7	7.6	16	14.4	23	11.3	203	100.0		Ref		
	No	85	92.4	95	85.6	180	88.7			2.05	0.83	5.54	0.134
Companion animals	Companion animal *												
	No	71	77.2	101	91.0	172	84.7	203	100.0		Ref		
	Yes	21	22.8	10	9.0	31	15.3			0.33	0.14	0.74	**0.008**
	Dog *												
	No	78	84.8	104	93.7	182	89.7	203	100.0		Ref		
	Yes	14	15.2	7	6.3	21	10.3			0.38	0.14	0.95	**0.044**
	Cat *												
	No	83	90.2	108	97.3	191	94.1	203	100.0		Ref		
	Yes	9	9.8	3	2.7	12	5.9			0.26	0.06	0.89	**0.046**
	Other												

	No	92	100.0	111	100.0	203	100.0	203	100.0		Ref		
	Yes	0	0.0	0	0.0	0	0.0			0.83	0.02	42.22	0.926
Health history	**HIV**												
	No	83	92.2	104	94.5	187	93.5	200	98.5		Ref		
	Yes	7	7.8	6	5.5	13	6.5			0.68	0.21	2.13	0.509
	Syphilis												
	No	78	86.7	91	83.5	169	84.9	199	98.0		Ref		
	Yes	12	13.3	18	16.5	30	15.1			1.29	0.59	2.90	0.533
	Hepatitis												
	No	85	93.4	99	90.0	184	91.5	201	99.0		Ref		
	Yes	6	6.6	11	10.0	17	8.5			1.57	0.57	4.74	0.391
	Cardiovascular diseases												
	No	68	73.9	76	68.5	144	70.9	203	100.0		Ref		
	Yes	24	26.1	35	31.5	59	29.1			1.30	0.71	2.43	0.396
	Tuberculosis												
													(CONCLUSÃO)
	No	81	88.0	97	89.0	178	88.6	201	99.0		Ref		
	Yes	11	12.0	12	11.0	23	11.4			0.91	0.38	2.20	0.834
	Diabetes												
	No	85	92.4	99	89.2	184	90.6	203	100.0		Ref		
	Yes	7	7.6	12	10.8	19	9.4			1.47	0.57	4.11	0.438
	Other diseases												
	No	78	85.7	94	85.5	172	85.6	201	99.0		Ref		
	Yes	13	14.3	16	14.5	29	14.4			1.02	0.46	2.25	0.958
Wash hands with water and soap	**Water and soap**												
	Yes	81	88.0	102	92.7	183	90.6	202	99.5		Ref		

	No	11	12.0	8	7.3	19	9.4			0.58	0.22	1.50	0.261

Overall, the proportions of shelter workers with previous or current infectious diseases were lower, with no reports of tuberculosis and equal percentages of HIV infection, syphilis, and hepatitis, amounting to 2.3% each (Table 2). However, no statistical differences were observed in the proportion of individuals who are homeless and shelter workers affected by HIV or hepatitis (p = 0.246 and p = 0.814, respectively). Likewise, cardiovascular disease and diabetes were reported by 25.3% and 6.9% of the shelter workers, respectively (Table 2), with no statistical difference from individuals who are homeless (p = 0.511 and p = 0.493, respectively). The bivariate analysis for the presence of anti-*SARS-CoV-2* IgG antibodies in individuals who are homeless showed statistically significant associations in this population (Table 1), where age (30 to 60 and >60 years old; p = 0.007 and p < 0.001, respectively) was associated with higher rates of IgG seropositivity, but tobacco use (p = 0.006), marijuana use (p = 0.003), and pet ownership (p = 0.008), including dogs (p = 0.044) and cats (p = 0.046) where associated with lower rates of IgG seropositivity (possible protective factors).In the bivariate analysis of the shelter workers, the presence of anti-*SARS-CoV-2* IgG antibodies was statistically associated with the city of origin (p = 0.025) and ethnicity (p = 0.015), where being from outside São Paulo and Black (compared to white) was associated with a higher seropositivity rate, as well as body pain (p = 0.013) and loss of sense of smell or taste (p = 0.001) as previous or current symptoms (possible risk factors).

In the multivariate analysis, a significantly increased risk of IgG seropositivity was observed in persons who are homeless of the age groups from 30 to 60 years old (OR 6.26, CI: 1.91– 20.56) and older than 60 years old (OR 10.88, CI: 2.35–50.44) when compared to individuals up to 30 years old (Table 3). In other words, adults and elderly individuals who are homeless were 6.26 and 10.88 times more likely to be IgG seropositive than younger individuals (up to 30 years old), respectively. Finally, tobacco use (OR 0.37, CI: 0.17–0.81), body pain (OR 0.34, CI: 0.13–0.87) as current or past *SARS-CoV-2* symptoms, and dog ownership (OR 0.22, CI:0.07–0.24) were all detected as protective factors for *SARS-CoV-2* exposure in persons who are homeless (Table 3).

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
													(CONTINUA)
Sociodemographic aspects	**Origin**												
	São Paulo	32	72.7	20	48.8	52	61.2	85	97.7		Ref		
	Others	12	27.3	21	51.2	33	38.8			2.80	1.15	7.07	**0.025**
	Age												
	Young (18–30 years-old)	9	19.6	6	14.6	15	17.2	87	97.7		Ref		
	Adult (31–60 years old)	35	76.1	34	83.0	69	79.3			1.46	0.47	4.76	0.516
	Elderly (> 60 years old)	2	4.3	1	2.4	3	3.5			0.75	0.03	9.68	0.829
	Gender												
	Male	24	52.2	20	48.8	44	50.6	87	100.0		Ref		
	Female	22	47.8	21	51.2	43	49.4			1.15	0.49	2.67	0.752
	Race/Ethnicity												
	White	21	47.7	9	21.9	30	35.3	85	97.7		Ref		
	Black	10	22.7	17	41.5	27	31.8			3.97	1.35	12.47	**0.015**
	Mixed (Pardo)	13	29.6	15	36.6	28	32.9			2.69	0.93	8.17	0.072
	Level of education												
AAAA													

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
(CONTINUAÇÃO)													
	Higher education	25	54.3	16	39.0	41	47.1	87	97.7	Ref			
	High school	16	34.8	21	51.2	37	42.5			2.05	0.84	5.14	0.119
	Elementary school	5	10.9	4	9.8	9	10.4			1.25	0.27	5.43	0.764
	Tabacco												
	No	33	71.7	34	85.0	67	77.9	86	98.9	Ref			
	Yes	13	28.3	6	15.0	19	22.1			0.45	0.14	1.28	0.145
	Marijuana												
	No	45	97.83	39	97.50	84	97.67	86	98.9	Ref			
	Yes	1	2.17	1	2.50	2	2.33			1.15	0.04	29.83	0.920
	Cocaine												
	No	46	100.00	39	97.50	85	98.84	86	98.9	Ref			
	Yes	0	0.00	1	2.50	1	1.16			3.53	0.14	89.16	0.444
	Crack												
	No	46	100.00	40	100.00	86	100.00	86	98.9	Ref			
	Yes	0	0.00	0	0.00	0	0.00			1.24	0.63	2.40	0.527
	Injetables												
	No	46	100.00	40	100.00	86	100.00	86	98.9	Ref			
	Yes	0	0.00	0	0.00	0	0.00			0.78	0.11	4.08	0.773
Use of face mask during pandemic	**Face mask use**												
	Yes	45	97.83	39	97.50	84	97.67	86	98.9	Ref			
	No	1	2.17	1	2.50	2	2.33			0.87	0.03	22.40	0.920

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
(CONTINUAÇÃO)													
Contact history	**Contact with someone positive**												
	No	23	51.11	13	34.21	36	43.37	83	95.4		Ref		
	Yes	22	48.89	25	65.79	47	56.63			2.01	0.83	4.98	0.124
Current or previous (last 3 months) COVID-19 symptoms	**Fever**												
	No	40	86.96	31	75.61	71	81.61	87	100.0		Ref		
	Yes	6	13.04	10	24.39	16	18.39			2.15	0.72	6.93	0.179
	Difficulty breathing												
	No	40	86.96	34	82.93	74	85.06	87	100.0		Ref		
	Yes	6	13.04	7	17.07	13	14.94			1.37	0.42	4.64	0.600
	Tiredness												
	No	33	71.74	32	78.05	65	74.71	87	100.0		Ref		
	Yes	13	28.26	9	21.95	22	25.29			0.71	0.26	1.89	0.500
	Body pain												
	No	40	86.96	26	63.41	66	75.86	87	100.0		Ref		
	Yes	6	13.04	15	36.59	21	24.14			3.85	1.38	11.98	**0.013**
	Throat pain												
	No	37	80.43	30	73.17	67	77.01	87	100.0		Ref		
	Yes	9	19.57	11	26.83	20	22.99			1.51	0.55	4.20	0.423

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
													(CONTINUAÇÃO)
	Diarrhea												
	No	42	91.30	35	85.37	77	88.51	87	100.0		Ref		
	Yes	4	8.70	6	14.63	10	11.49			1.80	0.48	7.52	0.391
	Chest pain												
	No	42	91.30	36	87.80	78	89.66	87	100.0		Ref		
	Yes	4	8.70	5	12.20	9	10.34			1.46	0.36	6.28	0.594
	Dry cough												
	No	39	84.78	28	68.29	67	77.01	87	100.0		Ref		
	Yes	7	15.22	13	31.71	20	22.99			2.59	0.94	7.67	0.073
	Loss of sense of smell or taste												
	No	42	91.30	24	58.54	66	75.86	87	100.0		Ref		
	Yes	4	8.70	17	41.46	21	24.14			7.44	2.43	28.20	**0.001**
	Headache												
	No	30	65.22	27	65.85	57	65.52	87	100.0		Ref		
	Yes	16	34.78	14	34.15	30	34.48			0.97	0.40	2.36	0.950
Previous COVID-19 test	**Previous COVID-19 test**												
	No	35	76.09	36	87.80	71	81.61	87	100.0		Ref		
	Yes	11	23.91	5	12.20	16	18.39			0.44	0.13	1.35	0.166

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
													(CONTINUAÇÃO)
Companion animals	**Companion animal**												
	No	24	53.33	21	52.50	45	52.94	85	97.7		Ref		
	Yes	21	46.67	19	47.50	40	47.06			1.03	0.44	2.44	0.939
	Dog												
	No	31	70.45	21	53.85	52	62.65	83	95.4		Ref		
	Yes	13	29.55	18	46.15	31	37.35			2.04	0.83	5.04	0.121
	Cat												
	No	34	77.27	35	89.74	69	83.13	83	95.4		Ref		
	Yes	10	22.73	4	10.26	14	16.87			0.39	0.11	1.36	0.139
	Others												
	No	42	95.45	39	100.00	81	97.59	83	95.4		Ref		
	Yes	2	4.55	0	0.00	2	2.41			0.22	0.01	4.62	0.326
Health history	**HIV**												
	No	45	97.83	40	97.56	85	97.70	87	100.0		Ref		
	Yes	1	2.17	1	2.44	2	2.30			1.13	0.04	29.07	0.934
	Syphilis												
	No	46	100.00	39	95.12	85	97.70	87	100.0		Ref		
	Yes	0	0.00	2	4.88	2	2.30			5.89	0.27	126.28	0.257
	Hepatitis												
	No	45	97.83	40	97.56	85	97.70	87	100.0		Ref		
	Yes	1	2.17	1	2.44	2	2.30			1.13	0.04	29.07	0.934

TABLE 2. DESCRIPTIVE STATISTICS AND RESULTS OF BIVARIATE ANALYSIS OF RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO, BRAZIL.

	Variables	Descriptive statistics								Crude OR	95% CI		p-value
		Negative		Positive		Total		Answer rate					
		n	%	n	%	n	%	n	%				
													(CONCLUSÃO)
	Cardiovascular disease												
	No	36	78.26	29	70.73	65	74.71	87	100.0		Ref		
	Yes	10	21.74	12	29.27	22	25.29			1.49	0.56	4.00	0.421
	Tuberculosis												
	No	46	100.00	41	100.00	87	100.00	87	100.0		Ref		
	Yes	0	0.00	0	0.00	0	0.00			1.52	0.82	2.82	0.183
	Diabetes												
	No	43	93.48	38	92.68	81	93.10	87	100.0		Ref		
	Yes	3	6.52	3	7.32	6	6.90			1.13	0.20	6.43	0.884
	Others												
	No	44	95.65	40	97.56	84	96.55	87	100.0		Ref		
	Yes	2	4.35	1	2.44	3	3.45			0.55	0.02	5.95	0.631
Wash hands with water and soap	**Water and soap**												
	Yes	44	97.78	39	95.12	83	96.51	86	98.9		Ref		
	No	1	2.22	2	4.88	3	3.49			0.44	0.02	4.80	0.513

There were no common risk or protective factors for IgG seropositivity between the homeless population and shelter workers. A higher risk for seropositivity was seen in Black shelter workers (OR 4.84, CI: 1.06–22.04) when compared to white shelter workers and in those that experienced loss of sense of smell or taste (OR 6.29, CI: 1.32–29.98) in the past months (Table 4).

TABLE 3. FINAL LOGISTIC MODEL FOR ANALYZING RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-SARS-COV-2 IGG IN 203 PERSONS EXPERIENCING HOMELESSNESS OF SÃO PAULO, BRAZIL

	Adjusted OR	**95% CI**		**p-value**
		Lower	**Higher**	
				(CONTINUA)
Age (Ref = Less than 30 years old)*				
From 30 to 60 years old	6.26	1.91	20.56	**0.003**
More than 60 years old	10.88	2.35	50.44	**0.002**
Gender (Ref = Male)				
Female	0.96	0.25	3.76	0.959
Others	0.00	0.00	Inf.	0.996
Race/Ethnicity (Ref = White)				
Black	2.48	0.84	7.31	0.099
Mixed (Brown—Pardo)	1.33	0.54	3.29	0.537
Indigenous Brazilian	14.25	0.58	352.70	0.105
Schooling level (Ref = Higher education)*				
Elementary school	0.52	0.12	2.21	0.377
High school	0.24	0.06	0.96	0.043

TABLE 3. FINAL LOGISTIC MODEL FOR ANALYZING RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-SARS-COV-2 IGG IN 203 PERSONS EXPERIENCING HOMELESSNESS OF SÃO PAULO, BRAZIL				
	Adjusted OR	**95% CI**		**p-value**
		Lower	**Higher**	
				(CONCLUSÃO)
Assistance by the government healthcare program (Ref = Yes)				
No	0.55	0.24	1.24	0.148
Tobacco (Ref = No)*				
Yes	0.37	0.17	0.81	**0.013**
Injectables (Ref = No)				
Yes	0.00	0.00	Inf.	0.990
Fever (Ref = No)				
Yes	2.48	0.92	6.63	0.071
Body pain (Ref = No)*				
Yes	0.34	0.13	0.87	**0.024**
Loss of sense of smell or taste (Ref = No)				
Yes	2.45	0.76	7.89	0.133
Dog owning (Ref = No)*				
Yes	0.22	0.07	0.74	**0.015**
Cat owning (Yes x No)				
Yes	0.22	0.03	1.49	0.122

TABLE 4. FINAL LOGISTIC MODEL FOR THE ANALYSIS OF RISK OR PROTECTIVE FACTORS ASSOCIATED WITH SEROPOSITIVITY OF ANTI-*SARS-COV-2* IGG IN 87 SHELTER WORKERS OF SÃO PAULO

	Adjusted OR	95% CI		p-value
		Lower	Higher	
Origin (Ref = São Paulo)				
Others	2.66	0.73	9.63	0.136
Age (Ref = Less than 30 years old)				
From 30 to 60 years old	0.85	0.20	3.70	0.829
More than 60 years old	0.37	0.01	9.19	0.541
Gender (Ref = Male)				
Female	0.33	0.09	1.19	0.092
Race/Ethnicity (Ref = White) *				
Black	4.84	1.06	22.04	**0.041**
Mixed (Brown—Pardo)	2.51	0.54	11.77	0.243
Body pain (Ref = No)				
Yes	2.75	0.68	11.20	0.157
Loss of sense of smell or taste (Ref = No) *				
Yes	6.29	1.32	29.98	**0.021**

4.5 DISCUSSION

The study herein reports a high seroprevalence of *SARS-CoV-2* infection in persons who are homeless and shelter workers from a large day-shelter in São Paulo city. The molecular results have ruled out active *SARS-CoV-2* infection at the time of sampling and suggest that the high observed seroprevalence may be a consequence of the exposure to the first wave of SARSCoV-2 in the city. With similar seroprevalence, both populations (homeless and shelter workers) were equally exposed to the virus, with a high probability that the daily agglomeration potentiated transmission at the shelter. The observed seroprevalence rates were significantly higher than the crude seroprevalence of 17.1% in blood donors from São Paulo observed a month later from this study (Sept 7th to 29th, 2020) [19]. The prevalence was also higher than reported worldwide; in a systematic review comprising 23 countries, including Brazil, the *SARS-CoV-2* seroprevalence in the general population varied from 0.4% (8/816) in Malaysia to 22.1% (117/528) in Iran as of August 2020 [20]. This finding reflects the current vulnerability individuals who are

homeless and related shelter workers were subjected to, corroborating observations that the pandemic response has amplified and deepened current inequalities [2].

The seroprevalence of *SARS-CoV-2* found in the homeless population also exceeds the crude seroprevalence rates reported in blood donors from the severely hit Amazon region from March to October 2020, which reported the highest prevalence in Brazil of 46.3% (422/911) in June 2020 [19]. A similarly high seroprevalence (~50%) was observed in a study comparing slums with no-slums households in Mumbai, India [21]. The present study corroborates with others, demonstrating that socioeconomic vulnerability, such as homelessness and living in slums, are risk factors for increased exposure to *SARS-CoV-2* [20] and demonstrates the importance of serosurvey to policies and decision-making strategies and pandemic preparedness.

The shelter workers evaluated in this study included healthcare and social assistance professionals, cooking, and maintenance personal. It has been well known that healthcare professionals are at a greater risk of exposure to *SARS-CoV-2* than the general population [22]. Strikingly, the seroprevalence of 47.1% (41/87) reported herein is higher than those of healthcare workers from other countries, ranging from 0 to 45.3% in a systematic review of 49 studies from North America, Asia, Europe, and Africa [23]. Notably, the prevalence rates vary according to the pandemic's timing and location, rate of participation, type of healthcare worker, direct contact with patients, demographics, and socioeconomic conditions, among others [24]. Despite such variations, the closest seroprevalence rate to this study was 45.3% (87/200) in frontline health workers in the U.K. at the peak of the first wave of pandemics [25]. Different from our study, the overall prevalence in the U.K. study included cumulative results from baseline (25% seropositivity) and follow-up (19.0%) and RT-qPCR positive results (21%) results. Exposure to the community and access to PPE were important risk factors for virus exposure. In our study, hundreds of people visited the shelter daily, and the workers did not have access to appropriate PPE such as N95 or surgical masks; they used personal homemade masks. These factors likely contributed to the high *SARS-CoV-2* seropositivity observed in the shelter workers herein.

Rates of seropositivity were expected to be higher than active infection (i.e., viral RNA detection via RT-qPCR) as antibodies remain present in serum after infection [26–28] and represent the cumulative exposure to *SARS-CoV-2* in the population. Also, significantly lower O. D. values for IgG titers were observed in persons who are homeless compared to shelter workers. Possible reasons for such difference may include the time of infection, COVID-19 disease severity [29], and/or health conditions (e.g., malnutrition, comorbidities, substance abuse, etc.). We also cannot discount the possibility of false negatives in the ELISA assay due to low antibody titers and immunological window, which could mean that this population was even more affected by the pandemic than the percentage estimated herein. Thus, serologic testing plays a critical role in understanding the *SARS-CoV-2* infection in different populations, and like in this study, helps identify segments of the population at a higher risk for infection.

Interestingly, active tobacco smoking status in individuals who are homeless was associated with a lower (protective) prevalence of *SARS-CoV-2* antibodies. It is still controversial if tobacco smoking reduces or increases the risk of contracting *SARS-CoV-2* and if smoking interferes with developing the more severe disease. There is documented evidence corroborating with the study herein, such as one study in Chicago shelters showing that individuals experiencing homelessness who were current smokers were less likely to be infected with *SARS-CoV-2* [30]. Similarly, studies in China and across Europe showed a lower prevalence of hospitalized current smoker patients than the general population [31,32]. Another study in France showed the protective effect of smoking in non-hospitalized and hospitalized patients and reported a lower probability of developing symptomatic or severe disease [33]. Similar studies in China showed a lower prevalence of smokers in patients with poor outcomes [34] and a protective effect against severe diseases associated with past smoking [35]. Many researchers have hypothesized that the anti-inflammatory effects of nicotine [32,36] or the nicotinic receptors [37] could play a protective role in the pathophysiology of COVID-19; these hypotheses have not been proven to date.

On the other hand, it is necessary to emphasize that several studies demonstrate the harms of smoking concerning the progression, increased vulnerability to severe disease, and worse outcome of COVID-19 [38,39], as well as other respiratory

disorders [40]. The most accepted explanation for the greater risk of severe disease is the increased ACE2 expression, the receptor implicated in virus-cell recognition in the bronchial epithelium [41–43]. This hypothesis was recently challenged by a study showing no significant mRNA expression levels of ACE2 receptors in individuals who never smoked compared with smokers [44]. Thus, such subject remains uncertain and beyond this study's scope. Most importantly, the WHO has emphasized the well-established tobacco use risks and strongly recommends tobacco cessation [45]. An interesting finding of our study was the fact that dog ownership by individuals who are homeless was identified as a protective factor against *SARS-CoV-2* infection. Most of the São Paulo city's sleep-in shelters do not have adequate space to accommodate pets in their facilities.

Thus, we speculate that persons who are homeless with dogs tend to agglomerate less than individuals without pets, justifying the protective factor. Also of interest was the fact that body pain was associated with lower seropositivity to *SARS-CoV-2*. Body pain is a symptom of *SARS-CoV-2* infection but also of many other tropical diseases that are common in Brazil, such as dengue, zika, and chikungunya fevers, and leptospirosis, as well as behavioral characteristics, such as drug use or mental health illness, and poor housing conditions. Thus, other diseases or conditions not assessed herein may have acted as confounding factors to this association. As expected, loss of sense of smell or taste had significantly increased the risk of seropositivity in shelter workers, in agreement with 440/567 (77.6%) individuals during the COVID-19 pandemic peak in London, who have also presented *SARS-CoV-2* antibodies [46].

The homeless population herein showed complex health histories, particularly associated with infectious diseases (HIV, syphilis, and tuberculosis). However, previous history of STDs and tuberculosis were not found to be associated with *SARS-CoV-2* infection. Despite growing concerns [47,48], it remains unknown whether people with previous history of tuberculosis, a respiratory disease, are more susceptible to *SARS-CoV-2* infection or the development of severe COVID-19 [48]. In this study, we did not evaluate latent TB infection or TB and SARSCoV-2 co-infection; and it may not be possible to rule out if people with previous TB history had worsened clinical symptoms.

Black shelter workers herein presented higher likelihood of being seropositive for SARSCoV-2 than white shelter workers, as previously shown that Black people were at higher risk of contracting COVID-19 than white people [49]. In Brazil, Black individuals have been reportedly more likely to live in poverty than white individuals, along with less access to basic needs, including healthcare [50]. Poverty-associated household and public transportation overcrowding hinder individuals' ability to protect themselves against *SARS-CoV-2* infection [51]. These findings highlight the existence of social inequalities in health for which the role of structural racism should be further studied, as previously reported in the USA [52–55]. Finally, despite the robust outcome found herein, the relatively low homeless sampling does not exclude confounding factors such as center employment, jobs with involuntary gathering such as cooking and cleaning (as opposed to social and medical assistances), and workers living on households of same neighborhood, which may have existed at the time and could partially or entirely produce the observed association herein.

This study has limitations. Although the analyzed homeless population showed similar sociodemographic characteristics (i.e., origin, age, gender, race/ethnicity, level of education) to a recent survey in São Paulo city [56], all participants are from a single region of the city. Thus, the results of *SARS-CoV-2* seroprevalence should not be extrapolated to the entire homeless population of São Paulo city. The lack of information about the proportion of individuals who are homeless of the Mooca subregion accessing this shelter at some point in time or daily also hampers our ability to extrapolate these results to the entire Mooca homeless population.

Future studies should include randomized, larger sample size, and better geographic representation. We cannot exclude, for example, the possibility that agglomerations in this day-shelter may have increased populational exposure to *SARS-CoV-2*, and thus, the same conditions may not apply to individuals who are homeless or shelter workers in other regions of the city. Additionally, all participants made self-declarations to the questionnaire related to their own history and perception of illness and symptoms, which may have led to inaccurate reports due to forgetfulness or lack of knowledge or understanding.

4.6 CONCLUSION

In conclusion, the present study reports a high *SARS-CoV-2* IgG seroprevalence in individuals who are homeless and related shelter workers from a day-shelter in São Paulo, Brazil. At the time of the study (August 2020), both homeless and social worker populations showed no active *SARS-CoV-2* infection, indicating that they were likely exposed sometime within the pandemic's first peak in the city. The homeless population of São Paulo has been exponentially increasing over the past years, and current socioeconomic and housing programs are not enough to lift individuals out of the streets.

Our study indicates that such living conditions led this homeless population to be severely affected by the pandemic. The effects of widespread infection were also not accounted for by official authorities, underscoring the importance of this study in providing the rationale needed to protect this population from the risk of infection by *SARS-CoV-2* amid new surges of the virus. We advocate for the accountability of the number of cases and deaths among individuals experiencing homelessness, targeted vaccination of this population, healthcare programs to shelters, diagnostic testing, and further investment in housing, cash transfer, and employment programs to attend individuals in vulnerable situations such as homelessness in the city of São Paulo. Our study has also shown significant risk and protective factors for *SARS-CoV-2* infection, including that Black shelter workers were at higher risk of *SARS-CoV-2* infection when compared with the white shelter workers. This finding indicates a difference in exposure according to race, providing evidence of race-associated health disparities for which the whole of structural racism should be further investigated.

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5. SEROSURVEY OF *ANTI-TOXOCARA CANIS* ANTIBODIES IN PEOPLE EXPERIENCING HOMELESSNESSAND SHELTER WORKERS FROM SÃO PAULO, BRAZIL

5.1 ABSTRACT

Despite being one of the most prevalent helminth parasitic zoonoses worldwide and particularly in socioeconomically vulnerable populations, toxocariasis remains to be fully investigated in persons experiencing homelessness. Accordingly, the present study has aimed to assess the seroprevalence and associated risk factors of Toxocara spp. spp. exposure in persons experiencing homelessness and shelter workers from a day-shelter in São Paulo city, Brazil. Anti-Toxocara spp. IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA). Univariable and multivariable logistic regression models were performed to assess the risks for toxocariasis. Overall, anti-Toxocara spp. IgG antibodies were detected in 89/194 (45.9%, 95% CI: 39.0–52.9%) persons experiencing homelessness, twice as high (OR=2.2; 95% CI=1.245–3.873; P=0.0089) than the frequency of 22/79 (27.8%, 95% CI: 19.2–38.6) in shelter workers. College education was the only protective factor for Toxocara spp. spp. exposure (OR: 0.23; P=0.018) revealed by logistic regression. Although indicating a multifactorial origin of toxocariasis, the present study has assessed a highly vulnerable population with high disease risks and premature death. Thus, the living conditions of the homeless population have

influenced the high prevalence of anti-Toxocara spp. antibodies verified here compared with domiciled shelter workers. Despite being less exposed, shelter and other outdoor workers may present an occupational risk to toxocariasis. Future studies should establish whether such environmental exposure might occur in persons experiencing homelessness in other regions worldwide.

5.2 BACKGROUND

Toxocariasis is considered one of the most frequent and relevant neglected parasitic zoonoses worldwide [1]. Toxocariasis is caused by the common roundworms *Toxocara canis* and *Toxocara cati*, whose definitive hosts are dogs and cats, respectively [2]. Humans become infected most commonly by accidental ingestion of embryonated *Toxocara spp.* . eggs present in contaminated food, water, or soil. An additional route of infection involves ingesting raw or undercooked viscera/meat of mammal or bird paratenic hosts harboring infective larvae (L3) [3]. Following ingestion, *Toxocara spp.* larvae from the eggs and/or tissues are released in the small intestinal lumen. They penetrate the intestinal wall and enter the circulation to be disseminated to different organs [4].

Despite usually resulting in asymptomatic chronic infection, this larval migration may cause severe disease, affecting various organs (visceral toxocariasis), eyes (ocular toxocariasis), and central nervous system (neurotoxocariasis) [5], depending on larval load, continuous reinfection, tissue distribution, and intensity of the host inflammatory response [4]. According to a recent meta-analysis, the overall global seroprevalence for toxocariasis has been estimated as 19% (95% CI: 16.6–21.4%; 62,927/265,327) [6], which is significantly higher in vulnerable socioeconomic groups [7]. Despite its high prevalence, gaps regarding serosurveys and risk factors in vulnerable populations, including homeless people, remain.

Among vulnerability factors, homelessness has emerged as an international human rights violation [8], representing a global phenomenon affecting both developed and developing countries [9, 10]. Homelessness may lead to serious health implications [11] mainly due to insufficient healthcare, inadequate nutrition, precarious living conditions, along with physical and mental illness [12]. In addition, health status may be aggravated by substance abuse and the long-term burden of chronic diseases, increasing morbidity and premature death risks compared with housed persons [13].

Among the Latin American countries, Brazil has the most unequal income distribution, with 6.5% (13.6 million people) of the nationwide population living in extreme poverty due to economic crisis and political disarray since 2014 [14]. Extreme poverty rose to an estimated 9.5% by late 2020, worsened by the COVID-19 pandemic, particularly in major urban settings [15]. Brazilian homelessness has grown around 140% since 2012, with over half (56.2%) living in southeastern Brazil, mainly in São Paulo [16].

Despite its high prevalence in vulnerable populations, toxocariasis in persons experiencing homelessness remains to be thoroughly investigated. Accordingly, the present study aims to assess the seroprevalence and risk factors associated with *Toxocara spp.* exposure in persons experiencing homelessness and shelter workers from the day-shelter providing care to this population in São Paulo city, Brazil, the city with the largest homeless population nationwide.

5.3 METHODS

5.3.1 Study area

This is a cross-sectional study of the population experiencing homelessness, and shelter workers, including healthcare and assistance professionals, such as nurses, social workers, administrative personnel, cooks, and cleaning and maintenance professionals who provide care to persons who are homeless. The study was conducted in São Paulo city (23°33′1″S, 46°38′2″W), the capital of São Paulo State, southeastern Brazil, the most populous city in Latin America, with 11,253,500 habitants at the time, a high Human Development Index (HDI) (0.805), and with a humid subtropical climate and average temperatures varying from 19 °C (winter) to 25 °C (summer) [17].

Sample collection was performed at a major shelter that serves the second-highest population experiencing homelessness in São Paulo City and accounts for 4779/24,344 (19.6%) persons experiencing homelessness in this town [17]. Tis shelter was a day-only public service center providing three meals a day and medical assistance to persons who were experiencing homelessness, with no dormitory or sleepover permission.

5.3.2 Sampling and questionnaires

Participants signed a consent form prior to sample collection. Serum samples were collected by venipuncture using commercial vacuum tubes (Vacutainer, BD Co., Curitiba, Brazil). Samples were centrifuged at 1295×*g* for 5 min, and the separated serum was stored at −20 ºC until further testing. The questionnaire for people experiencing homelessness collected information regarding sociodemographic aspects (city of origin, age, gender identity, self-identified race/ethnicity, and level of education), previous assistance by the government's healthcare program "Street Clinics," time being homeless, contact with dogs and cats, showering frequency, drinking water source/type, raw meat intake, contact with soil, and onychophagy.

All shelter workers were also interviewed with a structured questionnaire for sociodemographic, behavioral, and clinical information. For the shelter workers, questions were evaluated for sociodemographic aspects (city of origin, age, gender identity, self-identifed race/ethnicity, and level of education), contact with dogs or cats, drinking water source/type, raw meat intake, contact with soil, and onychophagy.

5.3.3 Enzyme- linked immunosorbent assay (ELISA)

Adult *T. canis* worms discharged in the feces of naturally infected puppies were collected. Adult female nematodes were exposed to 1% sodium hypochlorite for 5 minutes to remove surface debris, followed by washing with normal saline for 3 minutes. After washing, the anterior third of the worm was dissected to collect parasite eggs [18, 19].

Eggs of *T. canis* were incubated in 2% formalin for approximately 30 days at 28 °C to facilitate embryonation. Larvae were hatched and incubated at 37°C inserum-free Eagle's medium, according to the standard protocol [20]. The culture supernatant/medium containing the *Toxocara spp.* excretory–secretory (TES) proteins was removed at weekly intervals, and to this, 5 µl/ml of the protease inhibitor, phenylmethylsulfonyl fluoride (PMSF; 200 mM) was added. The TES proteins in the culture medium were concentrated using a commercial ultra-centrifuge filtration unit (Millipore, Danvers, MA, USA), dialyzed against distilled water, centrifuged (18,500×*g* for 60 min at 4 °C), and filtered using 0.22 µm membrane filters (Millipore). The protein concentration of the resulting TES fraction was determined as previously described [21]. Cross-reaction with other ascarids was avoided by pre-incubating

serum samples with *Ascaris suum* adult worm extract (AWE), following an established protocol [19]. Briefly, adult *A. suum* recovered from the intestine of slaughtered pigs were macerated in distilled water.

To this, one part of NaOH and nine parts of water were added, making a final concentration of 0.15 M. After incubation at room temperature for 2 hours, the pH of the material was neutralized with 6 M HCl and centrifuged at 18,500×*g* for 20 min at 4 °C. After removing the lipids with ether, the final supernatant was filtered through 0.22 µm pore-sized membrane filters (Millipore) to obtain the AWE. All serum samples used in TES-ELISA were pre-incubated for 30 min at 37 ºC with an AWE solution (25.0 µg/ µl) in 0.01 M phosphate-buffered saline (PBS, pH 7.2) containing 0.05% Tween 20 (PBS-Tween) (Sigma, St. Louis, MO, USA) at a dilution of 1:200. Polystyrene 96-well microtiter plates (Corning, Costar, NY, USA) were coated with TES proteins at a concentration of 1.9 µg/µl per well using the coating buffer, 0.06 M carbonate-bicarbonate buffers, at pH 9.6. The coating was accomplished by incubating these plates for 1 h at 37 °C and then for 18 h at 4 ºC. The plates were subsequently blocked for 1 h at 37 °C with 3% skimmed milk (Molico®, Nestle, Brazil) diluted in PBS-Tween. Serum samples pre-adsorbed with AWE were added to wells in duplicate, incubated at 37°C for 30 min, and washed three times with PBS-5% Tween. Anti-human immunoglobulin G (IgG; Fc-specifc) peroxidase secondary antibody (Sigma A6029, St. Louis, MO, USA) was added at a 1:5000 dilution and incubated for 45 min at 37 °C.

After an additional washing cycle, the peroxidase substrate o-phenylenediamine (Sigma*Fast*™ OPD, Sigma P9187, St. Louis, MO, USA) was added to the wells and incubated for 5 min to visualize the antigen–antibody reaction. The reaction was stopped by adding 2 N sulfuric acid and absorbance was measured at 492nm. Positive and negative controls were included in each plate. The cut-of value was determined as the mean absorbance of 96 negative control sera plus three standard deviations. Antibody levels were expressed as reactivity indexes (RI), which were calculated as the ratio between the absorbance value of each sample and the cut-of value.

Positive sera were titrated using the same ELISA methodology as described above [22], except that sera were diluted 1:200 in the *Ascaris* adsorbent, AWE, and

incubated for 30 min at 37 °C, then twofold serial dilutions were prepared (up to 1:12.800) using blocking solution and incubated for 60 min at 37 °C. The final titer of each serum sample was the highest dilution where a positive result was obtained.

Te avidity index (AI) of IgG was performed by a dissociation method, using a 6 M urea solution as the denaturing agent [23]. The AI, expressed as a percentage, was calculated as the mean optical density (OD) of (urea treated/urea-untreated) ×100. Values of AI up to 50 were considered low avidity (indicating recently acquired infection or recent toxocariasis), and AI values exceeding 50 were considered an indicator of high avidity (past toxocariasis).

5.3.4 Statistical analysis

Statistical analyses were performed using the programming language and free software environment R. Potential risk factors for toxocariasis were assessed by univariate analysis (Chi-square test or Fisher's exact test). Variables with a *P*-value≤0.2 in the univariate analysis were included in the multivariate analysis.

The model was adjusted using the Hosmer–Lemeshow test. The predictive performance of the final model was assessed by measuring the area under the receiver operator characteristic (ROC) curve. To increase the accuracy of the final model, predictive variables were tested for collinearity and the presence of influential observations [24, 25]. Multivariate logistic regression was used to assess the strength of the association between the presence of anti-*Toxocara spp.* antibodies and factors associated with toxocariasis and the results were expressed as odds ratios (ORs). Associations with a *P*-value<0.05 were considered statistically significant.

5.4 RESULTS

Overall, anti-*Toxocara spp.* IgG antibodies were detected in 89/194 (45.9%, 95% CI: 39.0–52.9) persons experiencing homelessness and 22/79 (27.8%, 95% CI: 19.2–38.6) in related shelter workers. The endpoint titers ranged from 400 to 6400. Seroprevalence in persons experiencing homelessness was statistically higher (χ2=6.834; df=1; OR=2.2; 95% CI=1.25–3.87; *P*=0.0089) than in workers from health and social shelters by the Chi-square test. According to the χ2 tests, associated risk factors, including gender (OR=1.4; CI=0.28–1.77; *P*=0.694), race/ethnicity (OR=1.1;

CI 95=0.57–1.95; *P*=0.994), previous assistance by the government's healthcare program "Street Clinics" (OR=0.6; 95% CI=0.30– 1.06; *P*=0.103), homelessness time (OR=0.78; 95% CI=0.37–1.66; *P*=0.349), contact with dogs (OR=1.1; 95% CI=0.43–2.73; *P*>1.0), cats (OR=0.8; CI=0.23– 2.80; *P*=0.998) or soil (OR=0.8; 95% CI=0.43–1.51; *P*=0.606), bathing frequency (OR=0.6; 95% CI=0.20– 1.44; *P*=0.322), availability of drinking water (OR=2.4; 95% CI=0.27–68.5; *P*=0.627), and onychophagy (OR=1.0 95% CI=0.46–1.95; *P*>1.0) were not statistically significant (Table 5). The only statistically significant associated risk factor for persons experiencing homelessness was having higher education, which was a protective factor against *Toxocara spp.* spp. infection (OR=0.2; 95% CI=0.06–0.72; *P*=0.018), as revealed by multivariate analysis (logistic regression) (Table 6).

TABLE 5: BIVARIATE ANALYSIS INCLUDING THE ASSOCIATED RISK FACTORS FOR ANTI-*TOXOCARA SPP.* SPP. ANTIBODIES IN PEOPLE EXPERIENCING HOMELESS IN SÃO PAULO CITY, BRAZIL (*N* = 194; POSITIVE = 89 AND NEGATIVE = 105)

Variable	**Positive (%)**	**Negative (%)**	**Odds Ratio (95% CI)**	**Statistical analysis**
				(CONTINUA)
City of origin				0.638
São Paulo city	30 (33.7)	31 (29.5)	Reference	
Others	59 (66.3)	74 (70.5)	1.2 (0.66–2.24)	
Gender identity				0.599
Female	8 (8.0)	13 (12.4)	Reference	
Male	81 (92.0)	92 (87.6)	1.4 (0.28–1.77)	
Age (Years)				0.112
> 60	17 (19.3)	14 (13.3)	Reference	
30 to 60	55 (62.5)	80 (76.2)	0.6 (0.25–1.26)	
< 60	16 (18.2)	11 (10.5)	1.2 (0.41–3.48)	

TABLE 5: BIVARIATE ANALYSIS INCLUDING THE ASSOCIATED RISK FACTORS FOR ANTI-*TOXOCARA SPP.* SPP. ANTIBODIES IN PEOPLE EXPERIENCING HOMELESS IN SÃO PAULO CITY, BRAZIL (*N* = 194; POSITIVE = 89 AND NEGATIVE = 105)

Variable	**Positive (%)**	**Negative (%)**	**Odds Ratio (95% CI)**	**Statistical analysis**
Educational background				0.051
Elementary school	47 (52.8)	44 (41.9)	Reference	
High school	38 (42.7)	46 (43.8)	0.8 (0.43–1.41)	
College	4 (4.5)	15 (14.3)	0.3 (0.07–0.79)	
Race/ethnicity				0.994
White	27 (30.3)	33 (31.4)	Reference	
Non-white	62 (69.7)	72 (68.6)	1.1 (0.57–1.95)	
Street clinics				0.10
				(CONTINUA)
No	62 (72.9)	63 (60.6)	Reference	
Yes	23 (27.1)	41 (39.4)	0.6 (0.30–1.06)	
Years of homelessness				0.349
< 1 year	23 (34.8)	26 (33.8)	Reference	
1–10 years	27 (40.9)	39 (50.6)	0.78 (0.37–1.66)	
> 10 years	16 (24.2)	12 (15.6)	1.5 (0.58–3.91)	
Drinking water				0.627
No	1 (1.18)	3 (2.97)	Reference	
Yes	84 (98.8)	98 (97.0)	2.4 (0.27–68.5)	
Raw meat intake				0.228

TABLE 5: BIVARIATE ANALYSIS INCLUDING THE ASSOCIATED RISK FACTORS FOR ANTI-*TOXOCARA SPP.* SPP. ANTIBODIES IN PEOPLE EXPERIENCING HOMELESS IN SÃO PAULO CITY, BRAZIL (*N* = 194; POSITIVE = 89 AND NEGATIVE = 105)

Variable	**Positive (%)**	**Negative (%)**	**Odds Ratio (95% CI)**	**Statistical analysis**
No	76 (90.5)	85 (83.3)	Reference	
Yes	8 (9.52)	17 (16.7)	0.5 (0.20–1.28)	
Contact with dog				1.0
No	79 (88.8)	94 (89.5)	Reference	
Yes	10 (11.2)	11 (10.5)	1.1 (0.43–2.73)	
Contact with cat				0.998
No	84 (94.4)	98 (93.3)	Reference	
Yes	5 (5.62)	7 (6.67)	0.8 (0.23–2.80)	
				(CONCLUSÃO)
Contact with soil				0.606
No	64 (72.7)	71 (68.3)	Reference	
Yes	24 (27.3)	33 (31.7)	0.8 (0.43–1.51)	
Onychophagy				1.0
No	69 (80.2)	81 (79.4)	Reference	
Yes	17 (19.8)	21 (20.6)	1.0 (0.46–1.95)	
Showering				0.322
Daily	82 (92.1)	91 (86.)	Reference	
Rarely	7 (7.87)	14 (13.3)	0.6 (0.20–1.44)	

Persons experiencing homelessness sampled herein were mainly 173/194 (89.2%) men, of which 81/173 (46.8%) were seropositive, whereas 21/194 (10.8%) were women and 8/21 (38.0%) seropositive. Mainly, 135/194 (69.6%) persons experiencing homelessness were between 30 and 60 years old, 133/194 (68.6%) were from another city than São Paulo, of which 59/133 (44.4%) were seropositive, while 61/194 (31.4%) were born in São Paulo and 30/61 (49.2%) seropositive. No signifcant differences were found between these groups.

Based on available health records at the shelter, 11/194 (5.7%) persons experiencing homelessness were seropositive to HIV, and 29/194 (14.9%) had syphilis. Anti-*Toxocara spp.* antibodies were observed in 2/11 (18.2%) HIV and 14/29 (48.3%) syphilis-positive individuals. No statistical association was revealed when comparing the presence of anti-*Toxocara spp.* antibodies and HIV $\chi 2$=3.51 df=1; OR:0.22; CI 95%=0.05–1.0; *P*=0.072) or syphilis ($\chi 2$=.28; df=1; OR: 0.74; CI 95%=0.38–1.65; *P*=0.597) seropositivity, by $\chi 2$ test.

TABLE 6: MULTIVARIATE ANALYSIS (LOGISTIC REGRESSION) INCLUDING RISK FACTORS FOR ANTI-*TOXOCARA SPP.* SPP. ANTIBODIES IN PEOPLE EXPERIENCING HOMELESS IN SÃO PAULO CITY, BRAZIL (*N* = 194; POSITIVE = 89 AND NEGATIVE = 105)

Variable	Positive (%)	Negative (%)	Odds ratio (95% CI)	Statistical analysis
Age (Years)				
> 60	17 (19.3)	14 (13.3)	Reference	0.280
30–60	55 (62.5)	80 (76.2)	0.6 (0.27–1.46)	0.816
< 60	16 (18.2)	11 (10.5)	1.1 (0.38–3.48)	
Educational background				
Elementary school	47 (52.8)	44 (41.9)	Reference	0.165
High school	38 (42.7)	46 (43.8)	0.6 (0.34–1.20)	0.018
College	4 (4.5)	15 (14.3)	0.2 (0.06–0.72)	
Street clinics				
No	62 (72.9)	63 (60.6)	Reference	
Yes	23 (27.1)	41 (39.4)	0.5 (0.28–1.03)	0.066

No risk factor was associated with seropositivity of anti-*Toxocara spp.* spp. antibodies and shelter workers, according to the statistical analysis (Additional file 1: Table S1).

5.5 DISCUSSION

To the authors' knowledge, this is the frst study conducting a serosurvey to detect anti-*Toxocara spp.* spp. antibodies and the associated risk factors among persons experiencing homelessness and revealed a high seroprevalence for anti-*Toxocara spp.* IgG (45.9%; CI 95%: 39.0–52.9%) in adults experiencing homelessness in São Paulo. Our study has also shown that persons experiencing homelessness were 2.2 times more likely to be infected than shelter workers, demonstrating a difference in toxocariasis exposure.

In Brazil, the seroprevalence of toxocariasis has been widely reported, ranging from 4.2% [26] to 63.6% [27] in children, and from 8.7% [28] to 71.8% [29] in adult populations. Recently, 58/280 (20.7%) pregnant women [30] and 212/328 (64.6%) inhabitants of a Brazilian traditional seashore population were seropositive for anti-*Toxocara spp.* antibodies [31]. Although 45.9% frequency herein has been substantially higher than the overall 27.5% (CI 95%: 14.8–42.3%) seroprevalence for toxocariasis in Brazil, results were similar to 141/306 (46.3%) adult blood donors living in the most populated city of northeastern Brazil [6], indicating a multifactorial cause for toxocariasis, as previously observed [32]. Nonetheless, the present study has assessed a highly vulnerable population, which has been associated with high risks o disease and premature death [13]. Tus, homeless living conditions may have influenced the high prevalence observed here compared with domiciled shelter workers.

Although several risk factors have been reportedly associated with toxocariasis, including being male, youngster, having contact with dogs, cats, soil, consuming raw meat, and drinking untreated water [6], none was statistically associated with the presence of anti-*Toxocara spp.* antibodies. Such outcomes may be due to low sampling or high sample heterogeneity, or more likely, the skewness of the homeless population, mostly males, adults, and reportedly drug users [33, 34]. Not surprisingly, the predominance of males in the homeless population observed here has already been reported in Canada (273/455; 60%) [35], Nicaragua (62/82; 75.7) (9), and Brazil (635/701; 90.7%) [36]. As the population studied here primarily represented adults between 30 and 60 years old, youth could not be adequately tested as an associated risk factor for toxocariasis. Besides being the largest city in Latin America, São Paulo

has been among the most multicultural cities worldwide, accounting for the largest population of migrants, immigrants, and refugees nationwide [37].

Such a scenario of social vulnerability has challenged health authorities, for example, seropositivity of anti-*Treponema pallidum* antibodies has been significantly higher (*P*=0.043) in
immigrant persons experiencing homeless conditions [38]. Fortunately, although the homeless population was predominantly composed of 133/194 (68.6%) migrants and 134/194 (69.1%) nonwhite persons, both variables were not associated with toxocariasis. Although pet ownership was not identified as an associated risk factor for toxocariasis, dogs and cats have been well established as the primary animal hosts for *Toxocara spp.* , particularly in developing countries where most cats and dogs have access to public parks and playgrounds, leading to soil contamination and human exposure to infective eggs [7]. Even though pet ownership, especially of dogs, has been reported among persons experiencing homelessness [39], in this study, only 21/194 (10.8%) individuals reported contact with dogs and 12/194 (6.2%) with cats. Interestingly, only 57/194 (29.4%) persons experiencing homelessness referred to having direct contact with soil, probably because their living areas within the eastern-urban setting of São Paulo city were covered mainly by concrete, asphalt, and/or cement.

As a limitation to the One Health approach, the study herein has not surveyed dog feces and soil for the presence of *Toxocara spp.* spp. eggs. Nevertheless, the lack of statistical significance of dog and cat ownership and soil contact, combined with high seropositivity to toxocariasis in persons who are homeless, may indicate high environmental exposure to infection. Tus, animal health interventions, including scooping pet feces and deworming dogs and cats (as well as all other owned and stray pets citywide), should be considered to mitigate the risk of environmental contamination by *Toxocara spp.*

In addition to environmental contamination, the ingestion of raw or undercooked meat or the viscera of paratenic hosts, including cows, pigs, and chickens, has been considered important risk factors for toxocariasis [40– 42]. In this study, only 25/194 (12.8%) homeless persons referred to ingesting raw meat, corroborating previous studies that indicated that access to fresh meat, fish, vegetables, and fruits by

persons experiencing homelessness was limited due to poverty conditions [43, 44]. Moreover, our research group has shown that persons experiencing homelessness in the same city of São Paulo were less likely to be infected by *Toxoplasma gondii* mainly due to consuming processed and ready-to-eat foods [34]. Tus, similar to toxoplasmosis, ingesting raw meat may represent a less important transmission route of toxocariasis for individuals who are homeless.

Te present study revealed that having a college degree was a protective factor for *Toxocara spp.* infection (OR: 0.23; *P*=0.018), corroborating that educational level has been a social determinant for human toxocariasis [6]. As previously shown, individuals with only high school education were more likely to be infected (OR=1.54) when compared with those with a college degree [45], and toxocariasis frequency was significantly higher in persons missing a high school degree [46, 47]. In addition to health and self-hygiene access and awareness, persons who are homeless and hold a college degree may have lived in better socioeconomic conditions before being houseless, reducing the exposure period to toxocariasis.

The occurrence of HIV (5.7%) and syphilis (14.9%) in the studied population corroborate other studies focused on high-risk sexually transmitted infections in persons experiencing homelessness [48, 49]; however, their presence was not associated with seropositivity for *Toxocara spp.* antibodies. A previous study has shown that being under treatment for HIV was significantly associated with toxocariasis (*P*=0.0087), and co-infection assessment was crucial to establish the synergism between HIV and tissue helminths [50].

Ethnic and racial disparities have also been associated with discrimination of persons experiencing homelessness [51], as these people have been more frequently associated with Black ethnicity [52]. Again, although high toxocariasis seropositivity has been linked to Black, nonHispanics, and other ethnic groups [44], 134/194 (69,1%) persons experiencing homelessness herein identified themselves as Black, and no statistical significance was found.

As a limitation, despite being considered the most widely employed test in toxocariasis serosurveys and diagnosis, the ELISA test has failed to differentiate between recent and chronic infection [53]. Here, IgG avidity was assessed to distinguish recent from past toxocariasis [23], and the avidity index indicated that all

ELISA-positive individuals had a past infection (high avidity>50). Tus, the presence of anti-*Toxocara spp.* antibodies was independent of the duration of homelessness. Another limitation in this study includes the possibility of biased memory precision given by the individuals responding to the sociodemographic questionnaire, where no precise inference could be made on the homelessness time frame. Nevertheless, 94/143 (65.7%) persons experiencing homelessness have declared to have been living for more than one year under homelessness conditions, supporting the long-term infection as detected by the avidity index. This study has also been limited by the difficulty in accessing individuals who were homeless, partially explained by the lack of studies involving such populations worldwide, mainly due to refusal to answer the sociodemographic questionnaire and blood sampling. Although such limitation may have impaired reliable outcome data to provide robust statistical analysis, the results have contributed to our understanding of toxocariasis in the homeless population.

Finally, questionnaire information to assess persons experiencing homelessness may be problematic, particularly regarding food intake and dietary habits, once such a population has often shown a chaotic lifestyle and a high prevalence of drug abuse and mental health disorders. Further studies should be conducted using higher sampling numbers and from different homeless populations worldwide to establish the exact impact of toxocariasis in such populations.

5.6 CONCLUSIONS

Tis is the frst study reporting a serosurvey of *Toxocara spp.* spp. antibodies in persons experiencing homelessness. Despite the limitations, our findings indicated that the frequency of anti-*Toxocara spp.* antibodies in persons experiencing homelessness was relatively higher compared with other populations. Besides educational level as a protective factor for toxocariasis, no other risk factor was associated with *Toxocara spp.* exposure in persons experiencing homelessness.

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6. ONE HEALTH APPROACH ON SEROSURVEY OF ANTI-*LEPTOSPIRA SPP*. IN HOMELESSPERSONS AND THEIR DOGS IN SOUTH BRAZIL

6.1 ABSTRACT

Although leptospirosis has been described as a worldwide bacterial zoonosis primarily affecting vulnerable populations, to date no study has focused on concomitant serosurvey of homeless persons and their dogs. The aim of the present study was, to use a One Health approach to serologically assess homeless persons and their dogs in 3 major cities of south Brazil (São Paulo, Curitiba, and Foz do Iguaçu). Environmental information was obtained with an epidemiological questionnaire given to all participants. A total of 200 human and 75 dog samples were tested for anti-*Leptospira spp.*spp. antibodies to thirty different serovars using

the microscopic agglutination test. None of the homeless persons were positive while 5 of the 75 (6.7%) dogs were positive. Among homeless population, 89% (177 of 200) were male, 61% (122 of 200) self-declared Non-white, and 67% (134 of 200) were educated up to the 8th school grade. Lower exposure of homeless persons to *Leptospira spp.* in the present study when compared to other vulnerable populations (slum and low-income residents) may be result of less direct exposure as they are able to rapidly change locations in response to flooding events. In addition, these results may reflect the effectiveness of a specific healthcare service provided to people living in the streets in the 3 cities. While dogs may be used as environmental sentinels for leptospirosis, the low seropositivity results found in this report may indicate low transmission risk to homeless owners in direct daily contact with their dogs.

6.2. INTRODUCTION

Leptospirosis, caused by a bacterium of genus *Leptospira*, is considered one of the neglected tropical diseases of public health importance worldwide [1]. Transmission may occur through contact with contaminated urine of sewer rats (*Rattus novergicus*) that serve as the main reservoir in urban settings, or through exposure to soil or water contaminated with *Leptospira spp.* [2]. This disease affects vulnerable populations in tropical countries due to socio-environmental issues including inadequate infrastructure and sanitation, water and soil contamination, garbage accumulation, and rodent proliferation; all of which are common scenarios in Brazilian slums [3,4].

Homeless populations worldwide live without adequate or permanent housing and have little access to public and private resources. These populations are susceptible to multimorbidity of infectious and mental illness, substance abuse, stigmatization, and interpersonal violence, and social exclusion [5]. In Brazil, the homeless population is estimated at 140,559 persons, primarily living in poor conditions of highly populated urban cities, along with 13,151 slums distributed in 734 cities of the 27 Brazilian states [6].

Dogs may share the same ecological bioaccumulation environment as owners and may act as sentinels or reservoirs for several zoonotic diseases [7]. Among these, leptospirosis may be life-threatening to both humans and dogs as it can lead to multiple organ involvement and death in severe cases [8]. Although the World Health Organization (WHO) has defined neighborhood dogs as semi-dependent on multiple families for living maintenance [9]. The role of stray dogs and dogs owned by

homeless persons in zoonotic diseases remains to be fully established, particularly in urban settings. Accordingly, the aim of this study was to serologically assess homeless persons and their dogs in Sao Paulo, Curitiba, and Foz do Iguaçu, 3 major cities of south Brazil.

6.3 MATERIAL AND METHODS

6.3.1 Study area and sample collection

The study herein was conducted in 3 major cities of south Brazil. Sao Paulo (23◦32′ 56″S; 46◦38′ 20″W) is the largest Brazilian (and Latin American) city with 12.4 million inhabitants; Curitiba (25◦25′ 42″S; 49◦16′ 24″W) is the ninth largest Brazilian city with 1.8 million inhabitants; and Foz do Iguaçu (25◦32′ 49″S; 54◦35′ 18″W) is the largest Brazilian border city (adjacent to Paraguay and Argentina) with 258,000 inhabitants. Homeless persons were contacted through local official health services and voluntarily participated by signed consent for themselves and their dogs. An epidemiological questionnaire was given to participants to assess environmental information while dogs were clinically examined. Municipality nurses collected blood from the people and certified veterinarians collected blood from the dogs. Serum samples were obtained by centrifugation, stored at − 20 ◦C, and tested by microscopic agglutination test (MAT) to detect anti-*Leptospira spp.*spp. antibodies, according to the Brazilian Ministry of Health [10] protocols. A collection of 30 serovars stored at 28 ◦C in Ellinghausen McCullough-Johnson-Harris media was used, including Andamana,Australis, Autumnalis, Bratislava, Bataviae, Bovis, Canicola, Castellonis,

Copenhageni, CTG, Cynopteri, Djasiman, Grippotyphosa, Guaricura, Hardjo, Hebdomadis, Icterohaemorraghiae, Javanica, Minis, Nupezo- 01, Panama, Patoc, Pomona, Prajtino, Pyrogenes, Sentot, Shermani, Tarassovi Whitcombi, Wolffi. The 1:100 dilution was used as the cutoff point. If a sample was positive for more than one serovar, the highest titer was considered the causative infection. The 1:100 dilution was considered the cutoff point to determined exposure to *Leptospira spp.*spp. as previous established [10]. If a sample was seropositive for more than one serovar, the highest titer was considered [11].

6.3.2. Statistical analysis

The data were tabulated, descriptive analysis was performed with the Epi Info version 7 statistical software (CDC, Atlanta, GA, USA), frequency distributions with 95% confidence intervals were calculated, and results were organized for presentation.

6.3.3 Ethical considerations

This research was approved by the Ethics Committee in Human Research at the Federal University of Parana (Register 80,099,017.3.0000.0102 and Protocol 3.166.749), Ethics Committee in Animal Use at the Federal University of Parana (Protocol 044/2016), Municipal Health Secretaries of Curitiba (Register 80,099,017.3.3002.0101 and Protocol 3.225.726) and Sao Paulo (Register 80,099,017.3.3004.0086 and Protocol 3,366,684). All were subordinated and approved by the National Human Ethics Research Committee of the Brazilian Ministry of Health. All research participants provided written informed consent.

6.4. RESULTS

Of the 200 humans samples, there were 119 (59.5%) homeless persons sampled in Sao Paulo, 59 (29.5%) in Curitiba, and 22 (11.0%) in Foz do Iguaçu. No human samples were positive for the presence of anti-*Leptospira spp.*. antibodies. While answers to the epidemiological questionnaire were obtained and gathered (Table 7), no associated risk factor was statistically significant due to the absence of seropositivity.

TABLE 7 - HUMAN EPIDEMIOLOGICAL DATA.

1) Demographic profile	Empty Cell	**n**	**%**
			(CONTINUA)
Curitiba		59	29.5
São Paulo		119	59.5
Foz do Iguaçu		22	11.0
	Total	200	100.0
Sex	Male	176	88.0
	Female	24	12.0
	Total	200	100.0
Race (self-declaration)	White	54	27.0
	Non-white	122	61.0

TABLE 7 - HUMAN EPIDEMIOLOGICAL DATA.

1) Demographic profile	Empty Cell	n	%
	Not rated	24	12.0
	Total	200	100.0
Age	<30 years old	42	21.0
	31–50 years old	81	40.5
	>50 years old	53	26.5
	Not rated	24	12.0
	Total	200	100.0
Educational	Up to 8th grade	134	67.0
Background	High School / University	40	20.0
	Not rated	26	13.0
	Total	200	100.0
City of origin	Local city	71	35.5
	Other cities	106	53.0
	Not rated	23	11.5
	Total	200	100.0
Travel to other cities	Yes	35	17.9
	No	128	64
	Not rated	37	18.5
	Total	200	100.0
2) Social profile			
Have seen rat			
	Yes	78	39.0
	No	79	39.5
	Not rated	43	21.5
	Total	200	100.0
Contact with family	Yes	91	45.5
	No	79	39.5

(CONCLUSÃO)

	Not rated	30	15.0
	Total	200	100.0
Resting placea	Street	89	44.5
	Shelter	31	15.5
	Hostel	106	53.0
	Not rated	19	9.5
	Total	245	
Pet owner	Yes	40	20.0
	No	130	65.0
	Not rated	30	15.0
	Total	200	100.0
Pet speciesa			
Dog owner		39	82.5

TABLE 7 - HUMAN EPIDEMIOLOGICAL DATA.

1) Demographic profile	Empty Cell	n	%
Cat owner		7	17.5
(Both)		6	15.0
	Total	40	100.0
Drug use	Yes	134	67.0
	No	39	19.5
	Not rated	27	13.5
	Total	200	100.0
Drug typea			
	Alcohol	85	42.5
	Tobacco	62	31.0
	Marijuana	53	26.5
	Cocaine	46	23.0
	Crack	35	17.5
	Other drugs	9	4.5
	Not rated	24	12.0
	Total	314	

Of the 75 dogs sampled, 41 (54.7%) were from Sao Paulo, 13 (17.3%) from Curitiba, and 21 (28.0%) from Foz do Iguaçu. All the dogs were adults of undefined breed. A total of 5 (6.7%) dogs tested positive for anti-*Leptospira spp.*spp. with a low variable serological titer (Table 8). Four dog samples reacted to Icterohaemorraghiae serovar (titer 100); one coreacted to Copenhageni (titer 100) and another to Pyrogenes (titer 200). The fifth positive sample co-reacted to Copenhageni (titer 1600) and Pyrogenes (titer 400). Results presented after exclusion of 35 of the 75 (46.7%) samples from dogs vaccinated against *Leptospira spp.*

TABLE 8. DISTRIBUTION AND FREQUENCY OF PRESENCE OF ANTI-*LEPTOSPIRA* ANTIBODIES DETECTED BY IFA IN DOGS OWNED HOMELESS PERSONS IN CURITIBA, FOZ DO IGUAÇU AND SÃO PAULO CITIES. RESULTS AFTER EXCLUSION OF 35/75 (46.67%) SAMPLES FROM VACCINATED DOGS FOR *LEPTOSPIRA* SPP.

City (State)	Dogs (n)	Titer	Frequency (%)
Curitiba - PR	13	0	–
Foz do Iguaçu - PR	21	0	–
São Paulo - SP	**41a**	0	–
		100	4/41 (9.7)
		200	2/41(4.8)
		400	1/41 (5.0)
		1600	1/41 (5.0)

Three dogs showed reactivity for more than one *Leptospira* spp. serovar.

6.5. DISCUSSION

To the authors knowledge, this study is the first epidemiological serosurvey to date of *Leptospira spp.*. in homeless persons worldwide. Previous studies were case reports of *Leptospira spp.*spp. infection in homeless or outdoor persons in London [12], Tokyo [13], Florida [14], Lisbon [15], and Marseille [16].

Although negative results for human leptospirosis in homeless persons of 3 major Brazilian cities was a pleasant surprise, human leptospirosis has sustained high endemic levels in Brazil, mostly in urban areas, with an average annual report of 3810 cases (1.9 cases/100,000), according to a recent 16-year (2000–2015) retrospective survey by the Brazilian Ministry of Health [17]. Sao Paulo State accounted for 11,884 cases, corresponding to 21% of the leptospirosis cases nationwide [17].

Furthermore, Sao Paulo City alone presented 2201 cases in a 10-year period (2007–2016) survey, mostly in males (82%), aged 20 to 59 years (65%), living in urban areas (86%), under flooding (39%) and in contact with rodents (36%) [18]. Interestingly, homeless persons in this study were also mostly males (88%), aged 31 to 50 years (41%), and reported contact with rats (39%). However, the westside region, where homeless in this study were sampled, has already presented the lowest overall leptospirosis frequency (7%) when compared to south (28%), east (24%) and north (19%) city regions [18]. Similarly, another study with 2000 interviews of homeless persons in Sao Paulo city have shown mostly men (86%), aged between 31 and 49 years (51%) [19], with over a half sleeping on streets instead shelters [20].

As a vulnerable and outdoor population, homeless people have greater environmental exposure to infectious diseases. Our research group has recently shown a 55% seropositive rate for COVID-19 among homeless persons in Sao Paulo, the highest prevalence worldwide at the time [21]. However, homeless people may be less directly exposed to leptospirosis, as they are able to rapidly change locations in response to flooding events. In comparison, residents of slums or low-income flooding areas who normally refuse to evacuate may be exposed to overflowing open sewers, which are 3-times more likely to contain pathogenic *Leptospira spp.* and

have 6-times more pathogen load [22] compared to areas with closed sewers. Thus, as floods in tropical countries may directly increase *Leptospira spp.*spp. infection and outbreaks [23], the lack of fixed housing may allow homeless persons to avoid infection by migrating within the city itself [24], and therefore decrease their exposure to the consequences of floods.

Despite the study herein has not surveyed environmental *Leptospira spp.*spp., a recent systemic review has shown that leptospiral organisms may grow into a biofilm in both nutrient-free and complex microbiota environments, remaining virulent for months, particularly in soils and sediments [25]. Thus, populations environmentally exposed to *Leptospira spp.*, such as homeless persons and their animals, are likely highly exposed in endemic areas such as the three surveyed cities of Sao Paulo, Curitiba and Foz do Iguaçu. Despite living outdoors, the homeless persons in this study had low (absent) environmental infection of *Leptospira spp.*, similar to lower seropositivity for *Toxoplasma gondii* of other vulnerable Brazilian populations [26]. In that study, 36% of homeless persons were seropositive for *T. gondii*, compared to 57% of people in a riverside community and 80% of indigenous individuals [26]. While homeless persons in this study were less exposed to bacterial *Leptospira spp.*spp. infection probably due to their ability to quickly relocation during flooding, their lower exposure to protozoan *T. gondii* infection was mostly attributed to habits of eating mainly processed food and lacking fresh vegetables and raw or undercooked meat in their daily diet. When considering vector-borne diseases, a previous study has indicated that homeless populations of Houston, Texas, USA may be at risk for Chagas disease, due to outdoors exposure to vectors including triatomines and blood-borne pathogen risk behaviors, such as drug use [27]. Further studies should be conducted to establish the occurrence of zoonotic pathogens among homeless persons, particularly those of environmental exposure.

The One Health approach herein has shown seropositive dogs with no concurrent human exposure, indicating that each pathogen may have different animal and environmental risks for causing human infection, and all should be surveyed together. In contrast, homeless persons are reportedly more exposed to diseases transmitted from human-to-human, such as sexually transmitted infections from unprotected sex and needle sharing behavior (syphilis, HIV, and hepatitis [28], and COVID-19 [21].

Despite the lack of positive results for leptospirosis in homeless persons in this study, lack of adequate water and sanitation should be considered intrinsic human rights [29]. In addition, housing itself has been a social determinant of health [30] and health equity [31]. Negative human leptospirosis in this study may also be attributed to the Brazilian Unified Health System in the 3 cities operating the Street Clinic (healthcare for people living in the streets), which attempts to improve access to health services, a lack of which may substantially increase homeless persons vulnerability [32].

Besides the human leptospirosis cases, all 3 cities also had confirmed dog cases of leptospirosis [33]. However, the 7% frequency of positive dogs we found was lower than the 11% of street and shelter dogs in Sao Paulo [34], and 14% of dogs from a slum area in Curitiba, Parana State [34]. In a downtown urban area of Parana State, 2% of owners and 21% of dogs were serologically positive [35]. Although the frequency we found for dogs may have been lower due to a relatively high vaccination rate (47% of dogs), results were corroborated by absence of seropositive homeless persons. In addition to positivity, one dog presented high titers (400 and 1600) to two serovars within serogroup Icterohaemorragiae, the same as previous studies mentioned [35,36].

As limitations in the present study, human and dog leptospirosis were solely surveyed by serological methods, as association of serology and molecular tests may improve sensitivity and specificity of leptospirosis [37]. Nonetheless, a comparative study of MAT and PCR in acute leptospirosis found similar detection results [38]. . In addition, samples were obtained through contact with local health services, which may have missed new, non-registered and homeless persons refusing health assistance. The high percentage of vaccinated dogs may have indicated a biased sampling of well-treated dogs and a well-assisted homeless population. Finally, despite being a multicentric study, relatively low sampling per city may impair extrapolation of these results to other homeless populations in Brazil or worldwide. As a complex disease, leptospirosis has reportedly affected mostly vulnerable and low-income populations with several socio-environmental issues [39], which includes homeless populations. Thus, further studies should be conducted to confirm our

results, including more sampling of homeless persons, other major cities in different regions, and different locations within each city.

In summary, lower exposure of homeless persons to *Leptospira spp.* in the present study when compared to other vulnerable populations (slum and low-income residents) may be the result of less direct exposure, as they are able to rapidly change locations in response to flooding events. In addition, such results may reflect the effectiveness of a specific healthcare service (Street Clinic) provided to people living in the streets in the 3 cities. While dogs may be used as environmental sentinels for leptospirosis, low seropositivity results in this study could indicate low transmission risk of direct daily contact with homeless owners.

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7. CONSIDERAÇÕES FINAIS

Diante dos resultados do Capitulo 1, bem como as informações trazidas por outras pesquisas realizadas sobre o tema, é notório que a população em situação teve uma maior exposição à pandemia de COVID-19. É importante ressaltar que devido a condição de vida dessas pessoas há uma imensa dificuldade em realizar as principais medidas de prevenção para infecção por SARSCov2. A preocupação com a saúde dessas pessoas é algo que já vem sendo relatado anteriormente e quando pensamos em um cenário de pandemia essa fragilidade cotidiana fica ainda mais evidente. Além da exposição, questões relacionadas à comorbidades e acesso a tratamento e prevenção, o que prejudica o segmento ainda mais.

O que ficou claro nesse capitulo e em outros trabalhos é que o fator aglomeração tem um impacto muito grande nessa situação. Ainda que o centro de acolhimento avaliado nessa pesquisa não era um local de dormitório nem permanência duradoura o fato de servir alimentação, acabava promovendo a aglomeração. Outros trabalhos aqui citados também trazem a permanência em abrigos como um forte fator de exposição ao vírus. Então mesmo com medidas adaptadas a intenção de contenção que houve parece não ter sido eficaz.

Para que essas pessoas possam evitar grandes aglomerações seja por busca de alimento, serviço ou repouso é importante oferecer um modelo de moradia individual e permanente que as possibilite superar a situação de rua. Um modelo já aplicado em alguns países que trouxe a opção de moradia permanente e individual foi o *Housing First*, que é uma metodologia que oferece a opção de moradia sem etapas excludentes ou pré-requisitos, afirmando a moradia como direito de todos e não um privilégio daqueles que merecem ou exclusivamente de quem possa pagar.

Esse modelo já trouxe resultados positivos como maior tempo de moradia fixa, diminuição do uso dos sistemas de saúde, reduções na exposição à violência, reduções no comportamento associado ao risco de infecção pelo HIV e pelo vírus da

hepatite C, menos overdoses acidentais e menor mortalidade geral entre pessoas que injetam droga (MACKINNON *et al.*, 2020 ;ONAPA *et al.*, 2021).

Além da própria população em situação de rua é importante ter maior atenção também aos profissionais que lidam diretamente com ela. Na condição de ter que se expor a presença do vírus é indispensável o uso de EPIs. A alta prevalência de infecção mostrada nessa população neste estudo pode ter relação direta com a falta de uso de EPIs, mas também é importante salientar que não houve isolamento social, uma vez que essas trabalharam sem pausa durante a pandemia, se expondo no transporte público, na rua e no trabalho. A condição de trabalho como um fator determinante de saúde precisa ser levada em consideração, proporcionando a esses indivíduos menos carga de trabalho ou a opção de um trabalho remoto sem a diminuição do seu salário.

Apesar das prevalências de leptospirose e toxocariase encontradas no capitulo 2 e no capitulo 3, é importante lembrar que não há outros estudos publicados referente a esses dados nessa população tonando precipitado dizer que estes não têm maior probabilidade de se infectar com esses patógenos. A hipótese de que por seu comportamento nômade possa ter evitado o contato com enchentes precisa de mais estudos que analisam outros fatores como, por exemplo: contaminação de solo em que essas pessoas permanecem e amostra randômica em regiões distintas das cidades.

Ainda assim os estudos de *Toxocara spp.* e *Leptospira spp.* aqui realizados trazem algo inédito e extremamente importante que é a preocupação do efeito da exposição a essas doenças nessa população. Além da necessidade de oferecer moradia adequada para essas pessoas para minimizar a exposição delas a sujidades como excretas de animais é importante também voltar o olhar para condição sanitária de cães que circulam nas ruas. Medidas como a desverminação e vacinação dos animais por si só já pode contribuir com a diminuição a transmissão de zoonoses (RANGEL *et al*., 2023) Além disso seria interessante o monitoramento com pesquisa sorológica e molecular, castração e prevenção para animais que estão sob a tutela de PSR, assim como para todos os outros.

E por último é de extrema importância a noção de guarda responsável e educação em saúde, não só da população em situação de rua, mas para sociedade

no geral. Tendo em vista que o abandono, descontrole populacional, falta de cuidado sanitário e negligência com bem-estar animal contribuem diretamente para a propagação de zoonoses (SANTANA & OIVEIRA, 2006)

8. CONCLUSÃO

Os dados relatados demonstram que a população em situação de rua precisa de maior atenção quando falamos de doenças com alto potencial contagioso. Juntamente com os funcionários que trabalham no centro de acolhimento, mostrando a necessidade de EPIs e de análise mais profunda de questões sociais em que essas pessoas estão inseridas. É também preciso realizar mais estudos relacionados zoonoses nesta população, para que sejam tomadas medidas necessárias para melhorar a relação homem-cão aqui existente. Para tanto, a oferta de uma moradia adequada e permanente se torna essencial para melhor condição sanitária dessas pessoas.

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10. ANEXOS

ANEXO 1 – APROVAÇÃO NO COMITÉ DE ÉTICA

SECRETARIA MUNICIPAL DA SAÚDE DE SÃO PAULO - SMS/SP

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Impacto de animais de companhia na saúde de populações humanas em situação de vulnerabilidade nos municípios de Curitiba, Paraná e São Paulo, São Paulo, Brasil.

Pesquisador: Alexander Welker Biondo
Área Temática:
Versão: 3
CAAE: 80099017.3.3004.0086
Instituição Proponente: Supervisão Técnica de Saúde Mooca / Aricanduva / Formosa / Carrão
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.602.513

Apresentação do Projeto:
-

Objetivo da Pesquisa:
-

Avaliação dos Riscos e Benefícios:

-

Comentários e Considerações sobre a Pesquisa:
Análise de emenda: Considerando os recentes relatos sobre a detecção de SARS-CoV-2 em animais de companhia e a grande proximidade deles com pessoas , principalmente cães e gatos, e a vulnerabilidade de pessoas em situação de rua, torna-se importante elucidar aspectos da história natural da doença, como o possível ciclo zooantroponótico SARS-CoV-2 em pets, em consonância com os preceitos do Sistema Único de Saúde. Visando assim contribuir para a pesquisa cientifica e consequentemente para resolução de problemas presentes hoje na sociedade de forma a acrescentar informações para esclarecer o comportamento epidemiológico da SARS-CoV-2. Para que novas coletas possam ser feitas, é necessário o adiamento do término do projeto com alteração do calendário. Além disso a inclusão de mais uma pesquisadora que está desenvolvendo

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ANEXO 2 – PUBLICAÇÃO ARTIGO COVID-19 CAP1

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

High SARS-CoV-2 seroprevalence in persons experiencing homelessness and shelter workers from a day-shelter in São Paulo, Brazil

Anahi Chechiado Couto[1☯], Louise Bach Kmetiuk[1☯], Ruana Renostro Delai[1], Ana Pérola Drulla Brandão[2], Cairo Oliveira Monteiro[3], Luciana Helena Antoniassi da Silva[3], Camila Soares[3], Alexandre Campos Banari[3], Renato van Wilpe Bach[4], Christina Pettan-Brewer[5], Andrea Pires dos Santos[6], Ana Marcia Sá Guimarães[3], Danielle Bruna Leal Oliveira[3], Edison Luiz Durigon[3], Alexander Welker Biondo[1*]

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Abstract

Brazil presents one of the highest COVID-19 death tolls in the world. The initial SARS-CoV-2 epicenter was São Paulo city. As of 2019, the homeless population of São Paulo city was estimated at 24,344 individuals, the largest national homeless population. The present study aimed to concomitantly assess the molecular and serological prevalence and associated risk factors of SARS-CoV-2 infection in a homeless population and related shelter workers from a day-shelter. Serum samples, nasopharyngeal and oropharyngeal swabs of persons who are homeless and shelter workers collected from August 25th to 27th, 2020 were tested for the presence of anti-SARS-CoV-2 IgM and IgG antibodies by ELISA and SARS-CoV-2 RNA by RT-qPCR, respectively. All swab samples tested negative by RT-qPCR. Seropositivity of IgM and IgG was 5/203 (2.5%) and 111/203 (54.7%) in persons who are homeless, and 5/87 (5.7%) and 41/87 (47.1%) in shelter workers, respectively, with no statistical differences between groups. The high seroprevalence found herein indicates early environmental and urban spreading of SARS-CoV-2, associated with sociodemographic and economic vulnerability.

Author summary

Brazil is one of the world's most social-economically unequal countries, with a rising homeless population potentialized by the SARS-CoV-2 pandemic, particularly in its largest city, São Paulo. While few studies, mostly in high-income countries, have addressed the impact of the coronavirus pandemic on homeless populations, none has been carried

ANEXO 3 – PUBLICAÇÃO ARTIGO TOXOCARÍASE – CAP2

Santarém et al. Parasites & Vectors (2022) 15:373
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Parasites & Vectors

RESEARCH Open Access

Serosurvey of anti-*Toxocara canis* antibodies in people experiencing homelessness and shelter workers from São Paulo, Brazil

Vamilton Alvares Santarém[1], Anahi Chechia do Couto[2], Susana Zevallos Lescano[3], William Henry Roldán[3], Ruana Renostro Delai[4], Rogério Giuffrida[5], Louise Bach Kmetiuk[5], Alexander Welker Biondo[2,4,5*], Sriveny Dangoudoubiyam[3] and Andrea Pires dos Santos[5]

Abstract

Background: Despite being one of the most prevalent helminth parasitic zoonoses worldwide and particularly in socioeconomically vulnerable populations, toxocariasis remains to be fully investigated in persons experiencing homelessness. Accordingly, the present study has aimed to assess the seroprevalence and associated risk factors of *Toxocara* spp. exposure in persons experiencing homelessness and shelter workers from a day-shelter in São Paulo city, Brazil.

Methods: Anti-*Toxocara* IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA). Univariable and multivariable logistic regression models were performed to assess the risks for toxocariasis.

Results: Overall, anti-*Toxocara* IgG antibodies were detected in 89/194 (45.9%, 95% CI: 39.0–52.9%) persons experiencing homelessness, twice as high (OR = 2.2; 95% CI = 1.245–3.873; *P* = 0.0089) than the frequency of 22/79 (27.8%, 95% CI: 19.2–38.6) in shelter workers. College education was the only protective factor for *Toxocara* spp. exposure (OR: 0.23; *P* = 0.018) revealed by logistic regression.

Conclusions: Although indicating a multifactorial origin of toxocariasis, the present study has assessed a highly vulnerable population with high disease risks and premature death. Thus, the living conditions of the homeless population have influenced the high prevalence of anti-*Toxocara* antibodies verified here compared with domiciled shelter workers. Despite being less exposed, shelter and other outdoor workers may present an occupational risk to toxocariasis. Future studies should establish whether such environmental exposure might occur in persons experiencing homelessness in other regions worldwide.

Keywords: Seroprevalence, Toxocariasis, Zoonosis, Homeless

Background

Toxocariasis is considered one of the most frequent and relevant neglected parasitic zoonoses worldwide [1]. Toxocariasis is caused by the common roundworms *Toxocara canis* and *Toxocara cati*, whose definitive hosts are dogs and cats, respectively [2]. Humans become infected most commonly by accidental ingestion of embryonated *Toxocara* spp. eggs present in contaminated food, water, or soil. An additional route of infection involves ingesting raw or undercooked viscera/meat of mammal or bird paratenic hosts harboring infective larvae (L_3) [3]. Following ingestion, *Toxocara* larvae from the eggs and/or tissues are released in the small intestinal

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One health approach on serosurvey of anti-*Leptospira* spp. in homeless persons and their dogs in South Brazil

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ABSTRACT

Although leptospirosis has been described as a worldwide bacterial zoonosis primarily affecting vulnerable populations, to date no study has focused on concomitant serosurvey of homeless persons and their dogs. The aim of the present study was, to use a One Health approach to serologically assess homeless persons and their dogs in 3 major cities of south Brazil (São Paulo, Curitiba, and Foz do Iguaçu). Environmental information was obtained with an epidemiological questionnaire given to all participants. A total of 200 human and 75 dog samples were tested for anti-*Leptospira* spp. antibodies to thirty different serovars using the microscopic agglutination test. None of the homeless persons were positive while 5 of the 75 (6.7%) dogs were positive. Among homeless population, 89% (177 of 200) were male, 61% (122 of 200) self-declared Non-white, and 67% (134 of 200) were educated up to the 8th school grade. Lower exposure of homeless persons to *Leptospira* spp. in the present study when compared to other vulnerable populations (slum and low-income residents) may be result of less direct exposure as they are able to rapidly change locations in response to flooding events. In addition, these results may reflect the effectiveness of a specific healthcare service provided to people living in the streets in the 3 cities. While dogs may be used as environmental sentinels for leptospirosis, the low seropositivity results found in this report may indicate low transmission risk to homeless owners in direct daily contact with their dogs.

1. Introduction

Leptospirosis, caused by a bacterium of genus *Leptospira*, is considered one of the neglected tropical diseases of public health importance worldwide [1]. Transmission may occur through contact with contaminated urine of sewer rats (*Rattus norvegicus*) that serve as the main reservoir in urban settings, or through exposure to soil or water contaminated with *Leptospira* spp. [2]. This disease affects vulnerable populations in tropical countries due to socio-environmental issues including inadequate infrastructure and sanitation, water and soil contamination, garbage accumulation, and rodent proliferation; all of which are common scenarios in Brazilian slums [3,4].

Homeless populations worldwide live without adequate or permanent housing and have little access to public and private resources. These populations are susceptible to multimorbidity of infectious and mental illness, substance abuse, stigmatization, and interpersonal violence, and social exclusion [5]. In Brazil, the homeless population is estimated at 140,559 persons, primarily living in poor conditions of highly populated urban cities, along with 13,151 slums distributed in 734 cities of the 27 Brazilian states [6].

Dogs may share the same ecological bioaccumulation environment as owners, and may act as sentinels or reservoirs for several zoonotic diseases [7]. Among these, leptospirosis may be life-threatening to both humans and dogs as it can lead to multiple organ involvement and death

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Serosurvey of *Trypanosoma cruzi* in persons experiencing homelessness and shelter workers of Brazil

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Although Chagas disease, caused by *Trypanosoma cruzi*, has been associated with social vulnerability worldwide, producing disability and mortality, no study to date has assessed this protozoal infection in persons experiencing homelessness. Accordingly, the present study aimed to assess anti-*T. cruzi* antibodies by Wiener Chagatest ELISA recombinant v.3.0 in serum samples of persons experiencing homelessness and related shelter workers in São Paulo, a city with reported vectors but no recent autochthonous case report. Overall, seropositivity to *T. cruzi* resulted in three of 203 (1.5%) persons experiencing homelessness and two of 87 (2.3%) shelter workers, with similar seroprevalence likely associated with their past social vulnerability. Although the seropositivity in persons experiencing homelessness and shelter workers was within 0 to 25.1% seroprevalence for chronic Chagas disease in the general Brazilian population, the disease has almost decreased 2-fold from the 1980s to 2000s, and such a wide range may not reflect the local disease status. In addition, the authors hypothesized that the similar seroprevalence and exposure between homeless persons and shelter workers herein may be more associated with shared past and present low-income social vulnerability than migratory movements, which may also include infection by sharing injecting drugs, vertical transmission, or blood transfusion. Thus, future studies are needed to confirm the active transmission of Chagas disease in São Paulo city. Moreover, Chagas disease should be considered as differential diagnosis in homeless persons and shelter workers, even in major disease-free Brazilian or other worldwide cities, mostly due to early exposure and vulnerable living conditions.

KEYWORDS
Chagas disease, epidemiology, health inequalities, homeless people, vulnerability

1. Introduction

Chagas disease, a protozoal anthropozoonosis caused by *Trypanosoma cruzi*, is mostly transmitted by feces and urine of infected triatomine bugs and is occasionally acquired by oral ingestion, transplacental congenital, and blood transfusion (1). Chagas disease has been included among the neglected diseases worldwide, with ~6–7 million people globally affected, with 20–30% cardiomyopathy and death rates (2). Originally restricted to rural endemic Latin American areas, Chagas cases have been since reported in several urban areas of the Americas including Canada as well as the African, Eastern Mediterranean, European,

and Western Pacific countries, associated with environmental changes and increased human mobility (3). In Latin America, established endemic areas have covered 21 countries, with ~100 million exposed individuals (2). In Brazil, chronic and asymptomatic disease forms may have been historically underdiagnosed as only acute Chagas cases have mandatory notification by the Ministry of Health (4). Nevertheless, chronic Chagas estimative may reach ~1.2–4.6 million Brazilians, causing 6,000 deaths per year (5).

Persons experiencing homelessness have been recognized as the most vulnerable populations worldwide, along with incarcerated persons, immigrants, abusive families, and refugees (6). The higher morbidity and less life expectancy in persons experiencing homelessness than the general population may be related to social inequality, absence of settled home associated with migratory movements, drug addiction, and mental health disorders (7). Vulnerable populations have also been associated with Chagas disease in several countries, particularly due to triatomine exposure, injecting drugs, and transplacental transmission, leading to high levels of disability and early mortality (7, 8).

Despite persons experiencing homelessness may be highly exposed outdoors to triatomine bugs and present higher risks of blood-borne and vertical transmission (9), no study to date has assessed anti-*T. cruzi* antibodies in such a population. Accordingly, the present study aimed to assess anti-*T. cruzi* antibodies in persons experiencing homelessness and related shelter workers of São Paulo city, southeastern Brazil.

2. Methods

The study herein was approved by the Ethics Committee in Human Health at the Brazilian Ministry of Health (CAAE: 80099017.3.3004.0086, protocol number: 3.366.684). This was a cross-sectional comparative serological study on people experiencing homeless and related shelter workers of São Paulo city, São Paulo state, southeastern Brazil. Although initially considered a control group, shelter workers were also a vulnerable and low-income population, with past and present exposure to Chagas vectors and disease, with public health importance on workers' health and occupational risk. Blood sampling was conducted in August 2020, during the COVID-19 pandemic when São Paulo was a pandemic world epicenter city, at a major day-only public service shelter, located in the eastern subregion, with the second highest city homeless population with ~5,000 individuals in 2020 (10). The shelter daily provided ~600 breakfast and 800 lunch meals at the time, with ~90 healthcare and assistance professionals providing direct care to homeless persons, as previously described (11).

Homeless people and shelter workers were invited to voluntarily participate and sign an official agreement form. Blood samplings were then performed by cephalic puncture; serum was obtained after centrifugation, stored at −20°C, and tested by using a commercial kit (Wiener Chagatest ELISA recombinant v.3.0, Wiener Laboratories SAIC, Argentina), using six purified recombinant antigens targets, with 99% sensitivity and 96% specificity to detect anti-*T. cruzi* antibodies, registered and approved by the Brazilian Ministry of Health.

3. Results

Overall, three of 203 (1.5%) persons experiencing homelessness and two of 87 (2.3%) shelter workers were seropositive to anti-*T. cruzi* antibodies. The three seropositive homeless persons were men, from 30 to 60 years old, mostly (2/3; 66.6%) born or lived previously outside São Paulo city, and identified themselves as brown color and "mixed-race Brazilians" with elementary school as the level of education, current alcohol and tobacco use, and unemployment as a reason for becoming homeless. No injecting drug use and history of sexually transmitted infections (STI) were mentioned by any seropositive homeless person. One individual reported a dry cough and fever. The two seropositive shelter workers were also 30 to 60 years old, one man and one woman, identified themselves as Black and white, with higher and elementary education, respectively. The seropositive woman reported current use of alcohol, tobacco, and cocaine, history of syphilis and hepatitis infection, chest pain, dry cough, and headache.

4. Discussion

To the authors' knowledge, this was the first worldwide serosurvey of *T. cruzi* in persons experiencing homelessness and correspondent shelter workers. Although the herein seropositivity in persons experiencing homelessness (1.5%) and shelter workers (2.3%) was within 0 to 25.1% seroprevalence for chronic Chagas disease in the general Brazilian population, overall the disease has decreased from 4.4% in the 1980s to 2.4% after 2000s and such a wide range may not reflect the local disease status (12). In addition, although the Chagas disease infection has been estimated in 1.0 to 2.4% of the Brazilian population (~1.9 to 4.6 million people) (13), acute cases of Chagas disease were reported in only 2/62 (3.2%) microregions of São Paulo state since 2008, with a relative risk of 0.01 in the São Paulo microregion in 2017 (14). Thus, in addition to potential autochthonous cases, such higher prevalence observed in the present study may be likely associated with socially vulnerable conditions and migration movements, as already established (8, 15).

As chronic Chagas disease has been mostly asymptomatic and based on the serological diagnosis, prevalence may be underestimated (7, 13). Nonetheless, Chagas disease was the main cause of death in the last 2 decades due to neglected tropical diseases in Brazil, with approximately a third of deaths occurring in a different state from the birthplace (16). In addition, individuals from other endemic Latin American countries may be infected before migration, as 28/633 (4.4%) Bolivian immigrants living in downtown São Paulo city were seropositive, epidemiologically associated with early rural laboring and known infected relatives in Bolivia (17).

As human migration has been indicated as a potential risk factor for Chagas disease, a Latin American problem may have turned into a global public health issue (18), particularly during the adaptation period, as migrants and refugees may likely experience vulnerability and homelessness. Not surprisingly, migrant groups stratified by birth country have commonly presented higher seropositivity when compared with nationwide endemicity (18).

In such a scenario, human migrations have been indicated as the critical factor for Chagas disease emergence and spreading in disease-free areas, mostly transmitted even in the absence of vector transmission by blood transfusion, unprotected sexual life and pregnancy leading to vertical infection, and organ transplantation (19).

In the study herein, while shelter workers were mostly born in the São Paulo city (61.2%), homeless persons were mainly (68.0%) from other Brazilian cities, reflecting the well-described migratory phenomena of vulnerable populations to major urban centers (11). The authors hypothesized that the similar seroprevalence and exposure between homeless persons and shelter workers herein may be more associated with shared past and present low-income social vulnerability than migratory movements, which may also include infection by sharing injecting drugs, vertical transmission, or blood transfusion. However, as no epidemiological questions were made to shelter workers about temporary housing or visits to endemic areas outside São Paulo city, further studies should be conducted to fully establish such epidemiological findings. The results of this study corroborated the findings of shared vulnerability between homeless and related healthcare shelter workers during the COVID-19 pandemic (11). As previously suggested, healthcare workers in both globally high- and low-resource settings were likely to face risks and vulnerabilities that were shaped by local social and health system factors (20). In addition, non-healthcare workers, including shelter workers in cleaning, cooking, and maintenance services herein, may be also exposed to particular zoonotic pathogens and infectious bioaerosols at the workplace, as already shown (21).

Interestingly, the entomological surveillance service at São Paulo city has reported several confirmed vectors within city limits including *Panstrongylus geniculatus*, *Panstrongylus megistus*, *Triatoma infestans*, and *Triatoma sordida* infected by *T. cruzi* from 1988 to 2007 (22). Although these triatomine species were reported in the southern city area near the Atlantic Rainforest fragments, with no reports of vector domiciliation (22), *P. megistus* was recently reported in an urban São Paulo city park surrounded by artificial ecotopes (23). In addition, 34/108 (31.5%) triatomine species were molecularly infected by *T. cruzi* from 2012 to 2017 in the São Paulo state, with only one statewide reported case in 2016, two in 2017, and none in 2018, 2019, and 2020 (24). In this surveillance, the feeding behavior of collected triatomine species has shown contact with birds, opossum, rodents, and human beings (24). As triatomine species have been reportedly adapted to both natural and domestic areas (25), vulnerable populations may be more exposed outdoors due to absence of settled home or risk at work, neither ruling out nor confirming that positive homeless persons and shelter workers reported herein may be autochthonous cases of São Paulo city. As no molecular follow-up was made to characterize and compare strains of positive persons and those found in local vectors, we can only hypothesize on the matter. Thus, future studies are needed to confirm the active transmission of Chagas disease in São Paulo city, considering entomological and molecular surveys of triatomine species for *T. cruzi* infection, along with occurrence of acute cases in human populations and molecular matching of strains.

As limitations, the relatively low sampling and overall seropositivity herein may generate insufficient data to provide a representative statistical description and analysis of associated risk factors. In addition, the sampled homeless and shelter work populations were from a single city region with no bug vector report and may not be extrapolated to the entire homeless and healthcare populations and environmental exposure of São Paulo city. As no autochthonous case was confirmed in São Paulo city to date, negative results were expected, and the prevalence of general population was used as a control. As a cross-sectional approach, the present study represents a picture of people experiencing homeless and related shelter workers in São Paulo city in August 2020 for Chagas disease exposure. Samplings were performed in August 2020 only due to COVID-19, when São Paulo was the pandemic world epicenter city; otherwise, city hall would not allow samplings, and homeless persons and shelter workers would not come for samplings. Despite being required as ideal for research, control group sampling at the time was denied and later on would not reflect the two exposed groups. Nonetheless, further studies need to include a non-shelter worker or experiencing homelessness control group with the same sociodemographic conditions. In addition, a larger and randomized sample size of groups, along with better geographic representation, particularly in areas with the confirmed environmental presence of infected vectors, genetically compared *T. cruzi* strains were found in vectors, animals, and infected persons.

In conclusion, this is the first *T. cruzi* serosurvey in persons experiencing homelessness worldwide, with 3/203 (1.5%) seropositive homeless persons and 2/87 (2.3%) shelter workers, with similar seroprevalence likely associated with their previous and current low-income social vulnerabilities. As several *T. cruzi*-infected vectors have been described within São Paulo city limits, seropositive homeless persons and city-born shelter workers reported herein can neither be ruled out nor confirmed as autochthonous Chagas cases. Moreover, Chagas disease should be always considered as a differential diagnosis in homeless persons and shelter workers, even in major disease-free Brazilian or other worldwide cities, mostly due to early exposure and vulnerable living conditions.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee in Human Health at the Brazilian Ministry of Health (CAAE: 80099017.3.3004.0086,

protocol number: 3.366.684). The patients/participants provided their written informed consent to participate in this study.

Author contributions

LBK, FBF, and AWB: conceptualization, writing the original draft, reviewing, and editing. LBK, ACC, and AWB: performed the samples collection. LBK and GG: performed the serological analyses. FBF: supervision. All authors have read and agreed to the final version of the manuscript.

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Conflict of interest

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

Publisher's note

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