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RESEARCH ARTICLE

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Influence of landscape structure on previous exposure to *Leptospira* spp. and *Brucella abortus* in free-living neotropical primates from southern Brazil

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Abstract

The environments in which neotropical primates live have been undergoing an intense fragmentation process, constituting a major threat to the species' survival and causing resource scarcity, social isolation, and difficulty in dispersal, leaving populations increasingly vulnerable. Moreover, the proximity of wild environments to anthropized landscapes can change the dynamics of pathogens and the parasite-host-environment relationship, creating conditions that favor exposure to different pathogens. To investigate the previous exposure of free-living primates in Rio Grande do Sul State (RS), southern Brazil, to the bacterial agents *Leptospira* spp. and *Brucella abortus*, we investigated agglutinating antibodies against 23 serovars of *Leptospira* spp. using the microscopic agglutination test and *B. abortus* acidified antigen test in primate serum samples; 101 samples from primates captured between 2002 and 2016 in different forest fragments were used: 63 *Alouatta caraya*, 36 *Alouatta guariba clamitans*, and 02 *Sapajus nigritus cucullatus*. In addition, the forest remnants where the primates were sampled were characterized in a multiscale approach in radii ranging from 200 to 1400 m to investigate the potential

Abbreviations: AAT, acidified antigen test; AICc, Akaike's information criterion; ΔAICc, AICc difference; CEVS-SES/RS, State Health Department Rio Grande do Sul; DNA, deoxyribonucleic acid; ICMBio, Chico Mendes Institute of Biodiversity; LLP/IPVDF/RS, Leptospirosis Laboratory of the Instituto de Pesquisas Veterinárias Desidério Finamor; Mapbiomas, Annual Mapping of Land Cover and Land Use in Brazil Project; MAT, microscopic agglutination test; NHP, nonhuman primates; OR, odd ratio; PCR, polymerase chain reaction; RS, Rio Grande do Sul; SISBIO, biodiversity authorization and information system; VIF, variance inflation factor; ∑wi, Akaike's weights.

relationship of previous exposure to the agent with the elements that make up the landscape structure. The serological investigation indicated the presence of antibodies for at least one of the 23 serovars of *Leptospira* spp. in 36.6% (37/101) of the samples analyzed, with titers ranging from 100 to 1600. The most observed serovars were Panama (17.8%), Ballum (5.9%), Butembo (5.9%), Canicola (5.9%), Hardjo (4.9%), and Tarassovi (3.9%); no samples were seropositive for *Brucella abortus*. Decreased forest cover and edge density were the landscape factors that had a significant relationship with *Leptospira* spp. exposure, indicating that habitat fragmentation may influence contact with the pathogen. The data generated in this study demonstrate the importance of understanding how changes in landscape structure affect exposure to pathogenic microorganisms of zoonotic relevance. Hence, improving epidemiological research and understanding primates' ecological role in these settings can help improve environmental surveillance and conservation strategies for primate populations in different landscapes.

KEYWORDS

Alouatta, brucellosis, fragmentation, landscape, leptospirosis, primates, *Sapajus*

1 | INTRODUCTION

Leptospirosis is a zoonotic disease widely spread across Latin American countries; it is caused by pathogenic bacteria of the genus *Leptospira* and is highly relevant to human and animal health (Adler & de la Peña Moctezuma, 2010; Bharti et al., 2003; Schneider et al., 2013). Saprophytic species of *Leptospira* in the environment and those considered pathogenic are currently divided into eight species according to DNA analyses: *Leptospira borgpetersenii, Leptospira inadai, Leptospira interrogans, Leptospira kirschneri, Leptospira meyeri, Leptospira noguchii, Leptospira santarosai, and Leptospira weilii* (LeFebvre, 2016). Nevertheless, classification according to their antigenic composition is still used, comprising 23 serogroups and over 200 described serovars (Cerqueira & Picardeau, 2009; Levett, 2001; Machry et al., 2010).

Leptospirosis affects susceptible humans and animals in tropical and subtropical regions, and it is considered endemic in Brazil; its transmission can increase due to adverse climatic events, anthropic changes, and invasion of environments (Cerqueira & Picardeau, 2009; Costa et al., 2015; Ko et al., 1999; Lau et al., 2010). In urban areas, its occurrence in humans is related to low socioeconomic conditions and garbage accumulation in peripheral regions (Brasil Ministério da Saúde Secretaria de Vigilância em Saúde, 2021; Santos et al., 2018). Nonetheless, the influence of the environment on leptospirosis outbreaks around the world is still not well understood (Schneider et al., 2013), and some environmental factors (e.g., increased rainfall intensity and flooding) may contribute to the spread of the disease via waterborne transmission, which is exacerbated in tropical regions during rainy seasons (Barcellos & Sabroza, 2001; Brasil Ministério da Saúde, 2019; Schneider et al., 2012). In addition, alkaline or neutral soils, areas of intensive agricultural production, and conditions that allow rodent proliferation and circulation are associated with the bacterium's survival and contribute to its maintenance in the environment (Acha & Szyfres, 2003; Barcellos et al., 2003; Schneider et al., 2012).

Countless mammalian species serve as reservoirs of *Leptospira* spp. serogroups and/or serovars in the environment, including carnivores, rodents, marsupials, and primates, that directly participate in maintaining and disseminating the agent in the environment, especially through urine (Acha & Szyfres, 2003; Cilia et al., 2021; WHO, 2003). In this sense, investigating the occurrence or exposure to *Leptospira* spp. in wild species can help epidemiological research and shed more light on its environmental circulation (Andersen-Ranberg et al., 2016; Grimm et al., 2020; Ullmann & Langoni, 2011). Additionally, bacteria of the genus *Brucella* are also important zoonotic agents with worldwide distribution that affect domestic/wild animals and humans, causing economic losses and negatively affecting public health (Boschiroli et al., 2001). Given this scenario, it is pivotal to research infectious agents in free-living nonhuman primates (NHPs) from a unique health perspective while considering their phylogenetic proximity to humans and the fact that they often cohabit areas with distinct degrees of human impact, which entails opportunities for interspecies transmission of pathogens (Devaux et al., 2019; Harper et al., 2013; Kowalewski et al., 2010; Zinsstag et al., 2005).

The primates that inhabit Rio Grande do Sul State (RS; southern Brazil) depend on arboreal environments to survive and occupy forest areas with a wide diversity of plant species (Estrada et al., 2018; Jerusalinsky et al., 2010; Overbeck et al., 2015; Ribeiro et al., 2009). The three species of occurrence in RS are present in distinct biomes, and two of them—the brown howler monkey (*Alouatta guariba* *clamitans*) and the capuchin monkey (*Sapajus nigritus cucullatus*)—are associated with the mixed ombrophilous, dense, and semideciduous seasonal forests in the Atlantic Forest, while the black howler monkey (*Alouatta caraya*) is present in forest fragments of the Pampa biome (Lokschin et al., 2007; Printes et al., 2001; Silva & Bicca-Marques, 2013; Slomp et al., 2014). Nevertheless, the profound transformations in these ecosystems resulting from intense anthropic activity jeopardize the fauna and flora species in these environments (Bicca-Marques et al., 2020; Cordeiro & Hasenack, 2009; Estrada et al., 2018; Overbeck et al., 2007).

A broader approach has been proposed from a perspective that includes landscape contributing to the presence or exposure to pathogens in the environment (Arroyo-Rodríguez & Fahrig, 2014; Bloomfield et al., 2020). The landscape, in this context, can be described as a land surface that has distinct land cover elements that make up its spatial structure (Dunning et al., 1992; Fahrig, 2005). Changes in the structure of landscape composition and elements, such as patch density, urban cover, or water cover, for instance, can increase pathogen contact with NHPs and alter the parasite-host-environment relationship (Arroyo-Rodríguez & Fahrig, 2014; Gillespie & Chapman, 2008; Niehaus et al., 2020).

Hence, given the relevance of *Leptospira* and *Brucella* as zoonotic agents in human populations and the susceptible animals exposed, as well as the potential influence of landscape elements that can contribute to their presence and maintenance in the environment, this study sought to identify the previous exposure of *A. caraya*, *A. g. clamitans*, and *S. n. cucullatus* to *Leptospira* spp. and *Brucella abortus*. In addition, it sought to analyze the different landscapes where these primates live to shed more light on the relationship of landscape elements that could contribute to exposure to the agents in the investigated environments.

2 | MATERIALS AND METHODS

2.1 | Target population and study area

This study was based on analyzing 101 blood serum samples from free-living native primates captured in 48 forest remnants distributed in 26 municipalities in RS (southernmost Brazil; Figure 1). We captured 48 males and 15 females of *A. caraya* (*n* = 63), 28 males and 8 females of *A. g. clamitans* (*n* = 36), and 2 males of *S. n. cucullattus*.

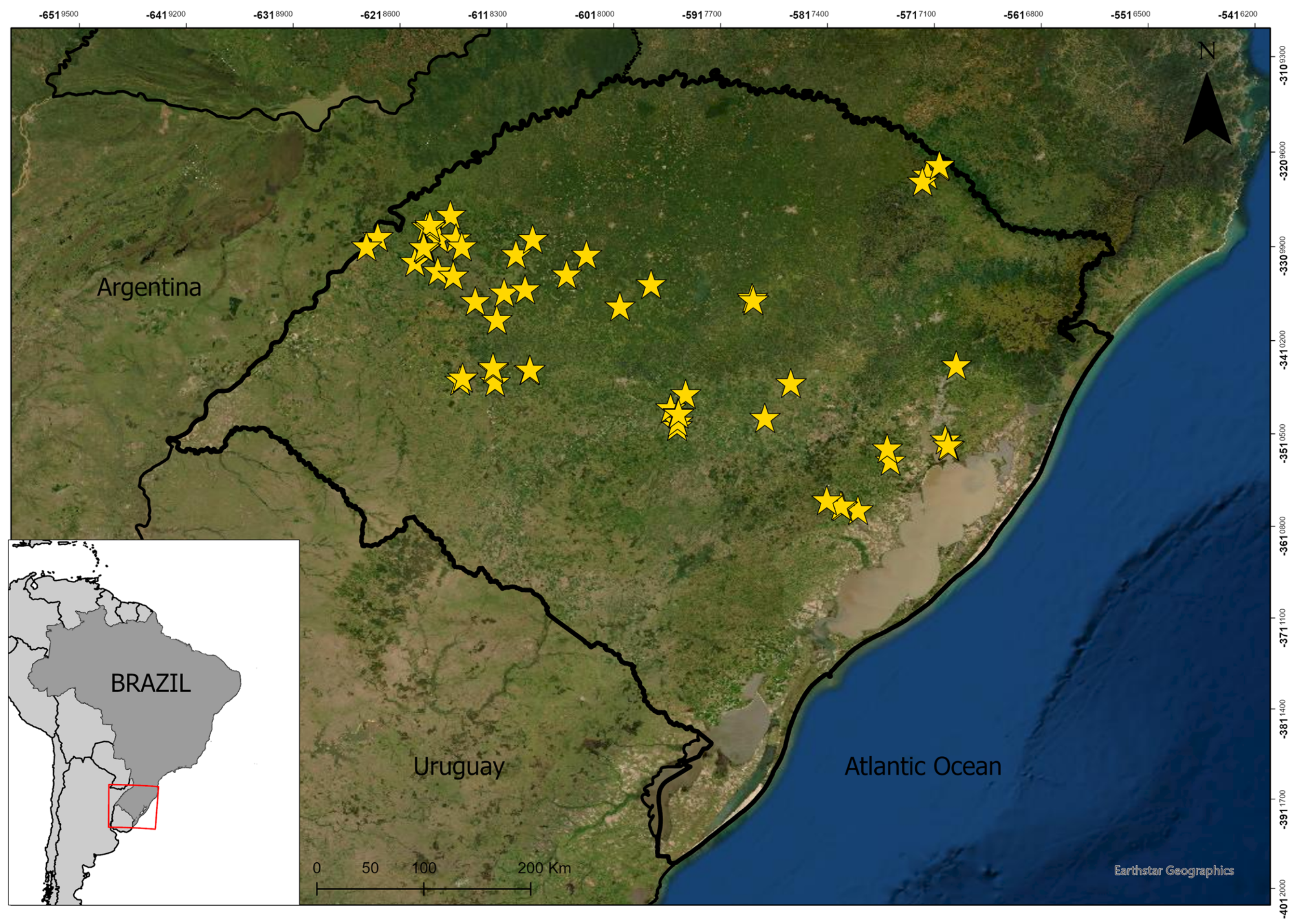

FIGURE 1 Distribution of points where native free-living primates were captured in forest remnants in Rio Grande do Sul State between 2002 and 2016. The figure was created using the ArcGis Pro® software.

The animals were captured between 2002 and 2016 by the State Health Department (CEVS-SES/RS) as part of the yellow fever monitoring program, and the animals were classified according to sex (male/female) and age group (juvenile/subadult/adult).

2.2 | Capture of individuals

The individuals were captured after chemical restraint with an association of 0.3 mg/kg levomepromazine, 0.5 mg/kg midazolam, and 10–15 mg/kg ketamine hydrochloride administered intramuscularly using a dart gun. All animals were identified with a transponder implanted in the dorsal region between the scapulae, and blood was collected via the brachial or femoral veins. After serum separation, the samples were identified and stored in cryotubes and kept frozen at −80°C in the CEVS-SES/RS until processed in the Leptospirosis Laboratory of the Instituto de Pesquisas Veterinárias Desidério Finamor (LLP/IPVDF-RS). The procedures were approved by the Chico Mendes Institute of Biodiversity (ICMBio) through the SISBIO authorizations related to the capture, collection, and processing of samples (13016-4, 13016-5, 67487-2, and 67487-3).

2.3 | Serological tests

Viable *Leptospira* spp. cultures in an enriched medium were used for the microscopic agglutination test (MAT) (Goris & Hartskeerl, 2014; OIE, 2021). The MAT is considered the standard serological method for the antemortem diagnosis of leptospirosis (Haake & Levett, 2015). The 23 *Leptospira* spp. serovars tested as antigens were: Andamana, Australis, Ballum, Bataviae, Bratislava, Butembo, Canicola, Castellonis, Whitcombi, Copenhageni, Grippotyphosa, Hardjo, Hardjo-bovis, Hardjo-bolivia Icterohaemorrhagiae, Javanica, Luis, Panama, Pomona, Pyrogenes, Sejroe, Tarassovi, and Wolffi. The antigens were kept in Sorensen liquid, and a 1:100 dilution of the samples was made as a cut-off point for screening; 200 µl of sample serum diluted in 800 µl Sorensen was used to obtain this dilution. From this dilution, 100 µl were transferred to tubes with 900 µl of Sorensen. Then, 50 µl of the second dilution was pipetted into polystyrene plates containing 96 wells. Subsequently, 50 µl of each of the 23 serovars tested were added. The plates remained 30 min at room temperature (23–25°C) until reading, which was performed under a dark-field microscope. Samples with at least 50% agglutination were considered positive in the screening. The serum samples that were positive in the screening were titrated in seven serial dilutions of ratio two (1:100 to 1:12,800).

For the rapid buffered acidified antigen test (AAT) for *B. abortus*, the antigen used was represented by whole cells in an inactivated suspension of *B. abortus* sample 1119-3 (Alton & Jones, 1969). The samples were homogenized with 30 µl of each serum and 30 µl of the antigen on a glass plate. After the serum and antigen were homogenized for 2 min, the reading was performed by evaluating agglutination using indirect light, and samples were considered positive when clumps were observed.

2.4 | Landscape characterization

Based on the georeferencing data of the primate capture site in the 26 municipalities, the landscapes were characterized using the fragment-landscape approach (Arroyo-Rodríguez & Fahrig, 2014; Galán-Acedo, Arroyo-Rodríguez, Estrada, et al., 2019). The central point of the fragment-landscape (centroid) at which the animal was captured was used as a reference so that buffers were produced at 200-m intervals, with radii ranging from 200 to 1400 m (Figure 2). A minimum radius of 200 m was determined due to howler monkeys' capacity to move long distances as they can travel roughly 1000 m daily, albeit they have lower movement rates between fragments in a nonforest matrix at distances above 200 m (Fortes et al., 2015; Mandujano & Estrada, 2005). We delimited the maximum radius at 1400 m as a form of balance to avoid spatial overlap between landscapes and not reduce the number of landscapes in the analysis.

Subsequently, land cover types were classified using satellite images with 30-m spatial resolution from the Annual Mapping of Land Cover and Land Use in Brazil Project (Mapbiomas, collection 5). The land cover maps developed corresponded to the year the primate samples were collected (between 2002 and 2016). The mapping of the Mapbiomas project has an accuracy of 85.8% for the Atlantic Forest biome and 85.7% for the Pampa biome. The following coverages were observed: forest formation, urban infrastructure, water cover (river, lakes, and/or oceans), agricultural cover (grassland formation + pastures + crops), rocky outcrops, planted forest, and wetlands. These variables were chosen because they have been shown to strongly influence primate movement patterns (Arce-Peña et al., 2019; Arroyo-Rodrígue, Moral, et al., 2013; Galán-Acedo, Arroyo-Rodríguez, Estrada, et al., 2019; Galán-Acedo, Arroyo-Rodríguez, Cudney-Valenzuela, et al., 2019). A detailed description of the landscape metrics used is listed in Table 1. Data processing was performed using the ArcGis Pro® and Fragstats® software (ESRI, 2014; McGarigal et al., 2012).

To define the permeability of the matrix around the forest fragments, we employed the ground cover functionality index for primates proposed by Galán-Acedo, Arroyo-Rodríguez, Estrada, et al. (2019). Thus, the area of each ground cover in the matrix was estimated and multiplied by a permeability gradient ranging from 1 (low permeability) to 6 (high permeability).

Next, the scale of the effect was determined, that is, the spatial scale of the landscape with the greatest explanatory power regarding exposure to pathogens (response variable). We followed the approach proposed by other researchers based on linear regressions between the fragment-landscape metrics at each of the seven spatial scales (200–1400 m, with 200 m intervals) using the pseudo-R^2 to estimate the force relationship between metrics and the response variable (Jackson & Fahrig, 2012, 2015; McGarigal et al., 2016). This perspective is important as the scale of the effect can differ between landscape metrics (Galán-Acedo et al., 2018). In Supplementary Information 1 and 2, the results of the scale of the effect between metrics and exposure to pathogens are presented.

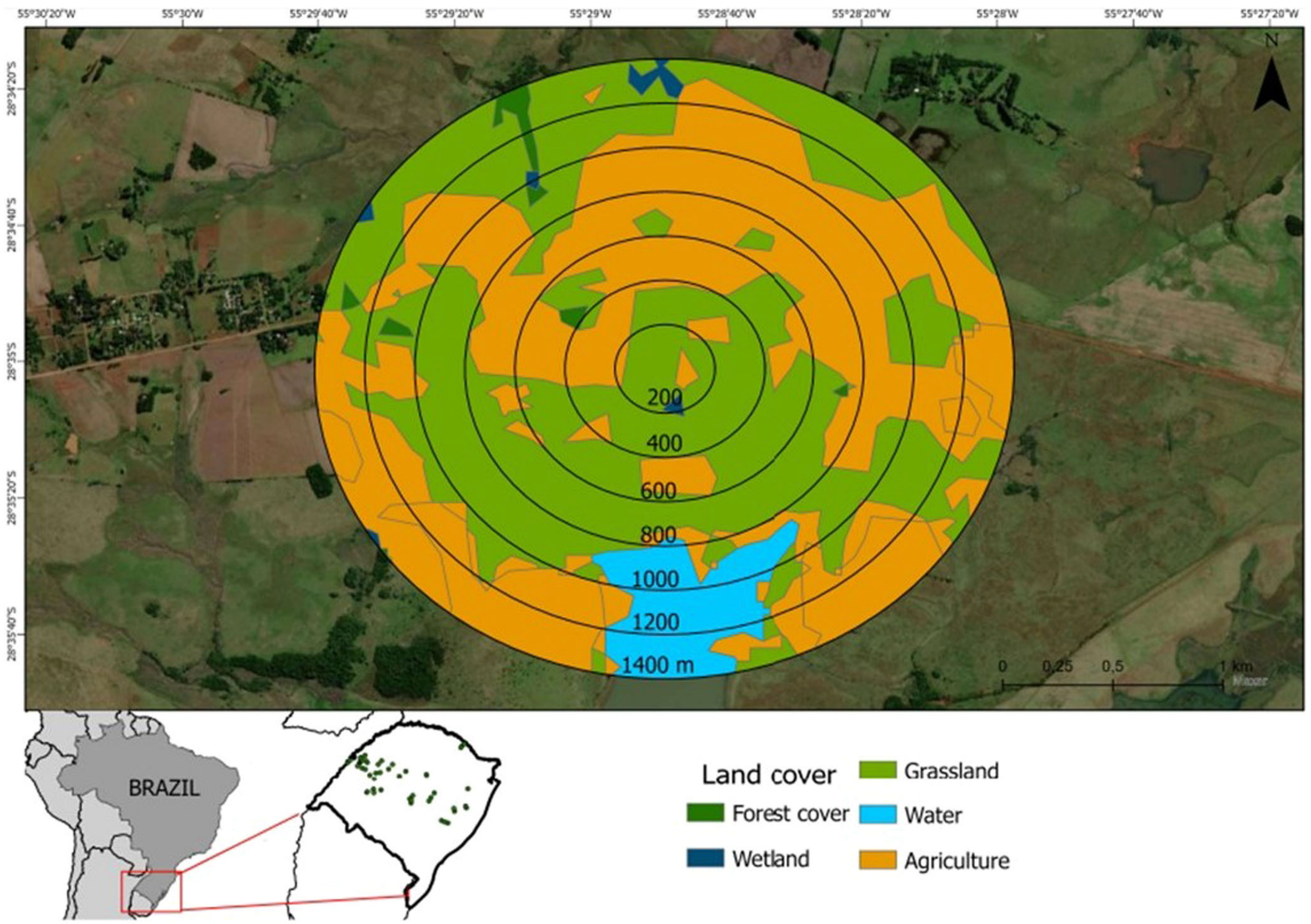

FIGURE 2 Fragment-landscape where a primate was captured using buffers with radii from 200 to 1400 m from the individual's sampling point. Land cover patterns in the matrix surrounding the fragment-landscape are illustrated in the figure.

TABLE 1 Landscape metrics used at the fragment-landscape scale in the forest fragments in which blood serum samples were obtained from *Alouatta caraya*, *Alouatta guariba clamitans*, and *Sapajus nigritus cucullatus* from different municipalities in Rio Grande do Sul State, Brazil, between 2002 and 2016.

Classification	Metric	Definition
Composition	Forest cover	Percentage of forest area in the fragment-landscape.
	Agricultural cover	Percentage of area with grasslands, pastures, and crops in the fragment-landscape.
	Water cover	Percentage of area with rivers, lakes, streams, ponds, and wetlands in the fragment-landscape.
	Urban infrastructure	Percentage of anthropogenic elements such as dwellings, roads, and bridges in the fragment-landscape.
	Matrix permeability	Percentage of each matrix land cover type weighted by its suitability for movement of arboreal primates in the fragment-landscape.
Configuration	Average Euclidean distance to the nearest fragment	Average shortest distances (m) between the edges of the forest fragments in the fragment-landscape.
	Fragment density	Number of forest fragments divided by the total fragment-landscape area.
	Edge density	The length of all forest edges in the fragment-landscape edges in the forest divided by the total landscape area (m/ha).

[a]Metrics based on Arce-Peña et al. (2019) and Galán-Acedo, Arroyo-Rodríguez, Estrada, et al. (2019), Galán-Acedo, Arroyo-Rodríguez, Cudney-Valenzuela, et al. (2019).

The variance inflation factor (VIF) was calculated using the "car" package in R 3.5.1 software to avoid bias regarding multicollinearity among landscape metrics previously measured at the effect scale (Fox & Weisberg, 2019; R Core Team, 2018). As such, only metrics that showed VIF < 4 were kept in the models, as VIF > 4 indicates possible collinearity, while a VIF > 10 indicates strong collinearity between the variables (Neter et al., 1996).

These preliminary analyses used generalized linear models using the "logit" distribution family to construct the binomial models of presence/absence of prior *Leptospira* spp. and *B. abortus* exposure. The dredge function was used to create all possible combinations between landscape metrics and agent exposure. We established each landscape variable's scale of effect measure in the models and included the number of individuals sampled in the overall model to determine sampling size bias. Landscapes with outliers (landscapes with the number of individuals sampled greater than 3) were identified with the betareg function.

To understand the effect of sampling size on the analysis, two modeling runs were performed, a "full analysis" (with all landscapes) and an "analysis without outliers." This approach was adopted to check the sampling size bias in the full analysis (number of individuals: minimum = 1; maximum = 19; mean = 2.5) and in the analysis without outliers (number of individuals: minimum = 1; maximum = 3; mean = 1.5). For each response variable, models were created that included all existing combinations between the predictor variables and the null model (containing only the intercept value) using the dredge function of the MuMln package in R software (Barton, 2016). Models were ranked using Akaike's information criterion (AICc) and corrected for small samples (Burnham & Anderson, 2002). Models were ranked from best to worst, with the model with the best fit being the one with the smallest AICc difference (ΔAICc). However, all models with ΔAICc < 2 were considered to be equally parsimonious (Richards et al., 2011). Last, the importance of each variable was verified using the sum of Akaike's weights (∑wi), and we estimated whether the relationship with the response variable was significant ($p \le 0.05$).

3 | RESULTS

3.1 | Serological tests

Of 101 serum samples from the NHPs analyzed by MAT for the presence of antibodies against 23 *Leptospira* spp. serovars, 36.6% (37/101) tested positive for at least one serovars, with titers ranging from 100 to 1600. Agglutination reaction was detected for 21 of the 23 serovars tested, with no positive samples observed for Castellonis and Andamana serovars. Positive reactions were observed in three individuals for serovars of the same serogroup: the Australis (Australis and Bratislava serovars) and Sejroe (Hardjo and Hardjo-bolivia ser; Hardjo-bovis and Hardjo-bolivia) serogroups. Only the serovar with the highest observed titer was considered in these cases. The serovars that presented the highest percentage of seropositive samples were Panama (17.8%), Ballum (5.9%), Butembo (5.9%), Canicola (5.9%), Hardjo (4.9%), and Tarassovi (3.9%). Among the positive samples, 54.1% were from *A. caraya*, 43.2% from *A. g. clamitans*, and 2.7% from *S. n. cucullatus*. The highest titer observed was 1600 for the Panama serovar in one *A. caraya* and two *A. g. clamitans* that had a titer of 800 for the Hardjo and Tarassovi serovars. Finally, one *A. g. clamitans* tested seropositive for 12 different serogroups. No sample was seropositive in the AAT for *B. abortus*. The overall results and titers observed for the different serogroups/sorovars are listed in Table 2.

3.2 | Relationship of fragment-landscape metrics to Leptospira spp. presence

In the "full analysis," which includes the outliers, seven models had delta AIC values of less than 2 and were considered good fits to the data (Supplementary Information S3). The best model contains the variables edge density ($\beta i = -0.045$; SE = 0.019; OR = 0.955; $p = 0.022$; Supplementary Information S5) and number of individuals sampled ($\beta i = 0.676$; SE = 0.452; odds ratio [OR] = 1.966; $p = 0.135$; Supplementary Information S5). The edge density variable was present in all seven models and showed a significant inverse relationship with *Leptospira* spp. occurrence (Figure 3). For each additional unit of edge density within the landscape, the odds of exposure to *Leptospira* spp. decreased by a factor of 0.955 (confidence interval [CI] = 0.919–0.993; i.e., for a one-unit increase in edge density, the odds of primates being exposed to *Leptospira* spp. decrease by 4.4%). All seven best models differed from the null model significantly ($p < 0.05$). The most important variables were edge density ($\sum w_i$ 0.79) and the number of individuals sampled ($\sum w_i$ 0.75).

In the "analysis without outliers," six models showed delta AIC < 2 (Supplementary Information S4). The edge density ($\beta i = -0.043$; SE = 0.019; OR = 0.957; $p = 0.022$; Supplementary Information S6) was again included in the best model, and the forest cover appeared in many parsimonious models (Supplementary Information S6), unlike the previous approach. The edge density variable again showed a significant inverse relationship with *Leptospira* spp. occurrence, indicating that it affected the occurrence of parasites even with the exclusion of outliers. The forest cover variable also showed an inverse relationship with *Leptospira* spp. exposure, unlike the previous approach in which forest cover did not have much importance on the occurrence of this agent (Figure 3; Supplementary Information S6). All parsimonious models differed significantly from the null model. The most important variables were edge density ($\sum w_i$ 0.69), followed by forest cover ($\sum w_i$ 0.55) and number of individuals ($\sum w_i$ 0.41). In the one top model where forest cover emerged as an important predictor (model 5, Supplementary Information S6), for each additional unit of forest cover within the landscape, the odds of exposure to *Leptospira* spp. decrease by a factor of 0.974 (CI = 0.952–0.997; i.e., for a one-unit increase in forest cover, the odds of primates being exposed to *Leptospira* spp. decrease by 2.5%). Complementary material with the complete modeling table of each

TABLE 2 Antibody titers to different *Leptospira* spp. serogroups/serovars detected in *Alouatta caraya*, *Alouatta guariba clamitans*, and *Sapajus nigritus cucullatus* from Rio Grande do Sul State, Brazil, from 2002 to 2016.

Agent		**Positive/total (%)**	***A. caraya***		***A. g. clamitans***		***S. n. cucullatus***	
***Leptospira* spp.**		**37/101 (36.6)**	**20/63 (37/7)**		**16/36 (44.4)**		**1/2 (50)**	
Serogroup	**Serovar**		**(+)**	**Titers**	**(+)**	**Titers**	**(+)**	**Titers**
Panama	Panama	18/101 (17.8)	8	100–1600	10	100–400	-	-
Ballum	Ballum	6/101 (5.9)	4	100	2	100–200	-	-
	Castellonis	-	-	-	-	-	-	-
Autumnalis	Butembo	6/101 (5.9)	2	100–200	4	100–200	-	-
Canicola	Canicola	6/101 (5.9)	3	100	3	100	-	-
Sejroe	Hardjo	5/101 (4.9)	2	100–200	3	100–800		
	Wolffi	2/101 (1.9)	-	-	1	100	1	100
	Hardjo-bolivia	2/101 (1.9)	2	100	1	100	-	-
	Hardjo-bovis	1/101 (0.9)	-	-	1	100	-	-
	Sejroe	1/101 (0.9)	1	100	-	-	-	-
Tarassovi	Tarassovi	4/101 (3.9)	2	100	1	800	1	200
Shermani	Luis	3/101 (2.3)	1	100	1	100	1	100
Celledoni	Whitcombi	3/101 (2.3)	1	100	2	100	-	-
Australis	Bratislava	2/101 (1.9)	1	100	1	100	-	-
	Australis	1/101 (0.9)	-	-	1	200	-	-
Pomona	Pomona	2/101 (1.9)	-	-	2	100	-	-
Bataviae	Bataviae	1/101 (0.9)	-	-	1	100	-	-
Gryppotyphosa	Gryppotyphosa	1/101 (0.9)	-	-	1	100	-	-
Icterohaemorragiae	Copenhageni	1/101 (0.9)	-	-	1	100	-	-
	Icterohaemorragiae	1/101 (0.9)	-	-	1	100	-	-
Javanica	Javanica	1/101 (0.9)	-	-	1	100	-	-
Pyrogenes	Pyrogenes	1/101 (0.9)	-	-	1	100	-	-
Andamana	Andamana	-	-	-	-	-	-	-

analysis (with and without the outlier) is available in Supplementary Information (S3 and S4).

4 | DISCUSSION

Given our findings, it is possible to confirm that, in the sampled environments, the native primates of RS *A. caraya*, *A. g. clamitans*, and *S. n. cucullatus* were previously exposed to distinct pathogenic *Leptospira* spp. serogroups/sorovars. Furthermore, landscape analysis showed that previous exposure to the pathogen was inversely related with the density of edges and forest cover in the sampled landscape fragments. These observations imply that the fragmentation of the sampled forest remnants influenced exposure to *Leptospira* spp. considering that these are two metrics related to environmental degradation (Arroyo-Rodríguez & Fahrig, 2014; Arroyo-Rodríguez, González-Perez, et al., 2013). Arboreal NHPs are particularly susceptible to the negative effects of fragmentation, and the impacts on their survival vary, including social isolation, decreased food resource supply, genetic drift, increased exposure to predators and hunting, and increased disease transmission (Altizer et al., 2003; Chapman et al., 2005, 2006; da Silva et al., 2015; Gillespie, Chapman, et al., 2005, Gillespie, Greiner, et al., 2005; Nunn et al., 2003). Thus, these findings are of the utmost importance because declining populations of NHPs worldwide due to fragmentation and exposure to one of the world's most prevalent zoonotic bacterial agents in tropical countries can jeopardize human populations and susceptible animals inhabiting the investigated settings.

No seropositive *B. abortus* samples were found, thereby corroborating other serological studies with free-living primates in Brazil (namely *A. caraya*, *Callithrix penicillata*, and *Sapajus flavius*) (Bueno et al., 2017; Molina et al., 2014). However, the

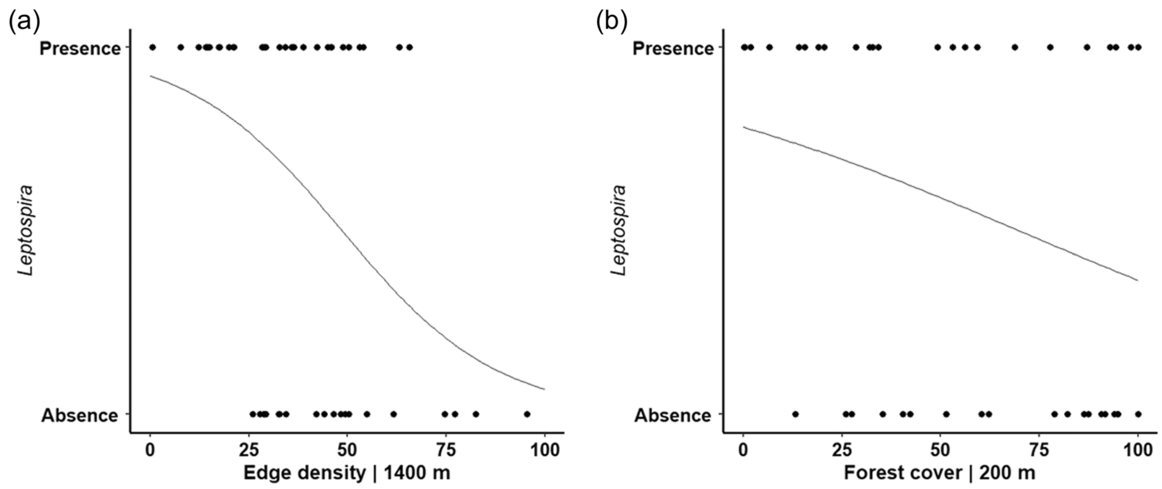

FIGURE 3 The relationship of landscape metrics with primate exposure to *Leptospira* spp. in the sampled landscapes. (a) Relationship between edge density and primate exposure to *Leptospira* spp. As edge density increases in the landscape, *Leptospira* spp. exposure decreases; (b) The relationship between forest cover and primate exposure to *Leptospira* spp. As forest cover increases in the landscape, *Leptospira* spp. exposure decreases.

epidemiological role of NHPs in this context still needs to be elucidated, given the possibility that they participate in the cycle as accidental hosts or as maintenance agents. The *Leptospira* spp. serovars with the highest number of positive samples in this study (i.e., Panama, Ballum, Butembo, Canicola, Hardjo, and Tarassovi) were previously isolated from domestic and wild species, considered reservoir hosts in the environment. In this study, the Panama serovar was revealed to have the highest number of seropositive samples and the highest titer observed in an *A. caraya* (1600) in the municipality of Santo Antônio das Missões (RS), which may be associated with acute infection (Pereira, 2005). This collection site was characterized as a small fragment adjacent to a rural property, with about 0.5 ha surrounded by crops. This serovar is considered pathogenic and has been described as belonging to the species *L. noguchii*, serogroup Panama, previously isolated from *Didelphis albiventris*, *Didelphis marsupialis*, and *Myocastor coypus*, and has been described in sheep in Brazil (Brenner et al., 1999; Cordeiro et al., 1981; Faine et al., 1999; A. S. A. Silva et al., 2007; É. F. Silva et al., 2009).

Regarding the other serovars observed more frequently in this study, Ballum has been isolated from some species of marsupials and wild rodents (Blasdell et al., 2019; Cordeiro et al., 1981; Lins & Lopes, 1984; Santa Rosa et al., 1975), and the serovar Tarassovi has been associated with pigs and wild boars (Bertelloni et al., 2020; Cilia et al., 2020). Cattle act as maintenance hosts for the Hardjo serovar (Ellis et al., 1981), while the Canicola serovar is associated with domestic dogs (Schuller et al., 2015), acting as sources of infection in farms (Barcellos et al., 2003). The Butembo serovar has been described in cattle and fallow deer (*Ozotocerus bezoarticus*) in Santa Catarina and Mato Grosso do Sul States, respectively (Saldanha et al., 2007; Vieira et al., 2011), in addition to being potential sources of infection for NHPs. What is more, numerous serogroups/sorovars traditionally recognized in domestic hosts have been isolated from wild hosts, including canids, cetaceans, cingulata, afrosoricidal, chiropterans, primates, in addition to reptiles and amphibians (Bertelloni et al., 2019; Cilia et al., 2021; Silva, Loffler, Brihuega, et al., 2016, Silva, Loffler, Santos, et al., 2016). Thus, various reservoir hosts may contribute to primate exposure in the natural environment.

In free-living primates, serological surveys have evidenced exposure to the agent. Girio et al. (2020) observed the serogroups Icterohaemorrhagiae, Canicola, and Autumnalis with higher frequency in *Cebus apella nigritus* in Ribeirão Preto (São Paulo State, southeastern Brazil), although the attempt to isolate the agent in urine samples did not detect the presence of *Leptospira* spp. DNA by polymerase chain reaction (PCR) assay. In another serological study with samples from 370 golden-headed lion tamarins (*Leontopithecus chrysomelas*) in Niterói (Rio de Janeiro State, southeastern Brazil), Molina et al. (2019) only found antibodies in two animals against the serovars Shermani and Hebdomadis. On the other hand, Molina et al. (2014) did not identify seropositive samples in *A. caraya* and *Callithrix penicillata* in São Paulo (São Paulo State), as did Bueno et al. (2017) when investigating 24 serovars in blood serum of *Sapajus flavius* in Santa Rita (Paraíba, northeastern Brazil). High prevalence associated with proximity to urban environments or near human settlements was observed in some families of NHPs, including callitrichids (36.8%), cercopithecids (28.8%), and cebids (20.4%), small- and medium-sized species with omnivorous diet (Andersen-Ranberg et al., 2016).

However, records of NHP showing clinical signs in the wild have been rare in different regions and under environmental conditions (Wilson et al., 2021). One study reported a black-tufted marmoset (*Callithix penicillata*) that died in an urban area in the Federal District (central Brazil) that was diagnosed with leptospirosis caused by *L. interrogans* without any serogroup and serovar being identified (Wilson et al., 2021). Isolation of *L. borgpetersenii* through urine sample was also documented in a healthy tufted capuchin (*Sapajus* apella) in Mato Grosso State (west-central Brazil) (Silva, Santos, et al., 2016). On the other hand, the investigation of *Leptospira* spp. DNA through PCR in urine samples of 50 tufted capuchin monkeys (*Sapajus apella nigritus*) did not expose the presence of the microorganism (Girio et al., 2020). Hence, data regarding the detection of the pathogen in free-living NHPs in Brazil are scarce.

In the landscape fragments where primates were sampled, the reduced forest cover and increased edge density were elements of the landscape structure associated with previous exposure to *Leptospira* spp. From a landscape perspective, forest cover indicates the amount of habitat, while edge density shows the fragmentation of forest remnants (Fahrig, 2003). Thus, our findings suggest that the loss and fragmentation of habitats influence the risk of exposure to the agent. In addition, we found a discrepancy in the strength of the relationship between forest cover (200 m) and edge density (1400 m) concerning exposure to *Leptospira* spp, showing how these metrics can operate at different scales and magnitudes. Other researchers have proposed that these two metrics act at different spatial and temporal scales in the life of primates, which enables various ecological processes to be modulated (Ewers & Didham, 2006; Fahrig et al., 2019; Galán-Acedo et al., 2018; Martin, 2018; Miguet et al., 2016; Suárez-Castro et al., 2018; Thogmartin & Knutson, 2007). In this sense, the relationships between landscape exposure and the pathogen depend on the spatial extent to which the landscape variables are measured, and the approach in its scale of the effect has been proposed to measure precisely how these elements can be related. Thus, we sought to identify the ideal scale, that is, the spatial extent in which the landscape element-exposure to the pathogen relationship is strongest, known as the scale of the effect (Crouzeilles & Curran, 2016; Jackson & Fahrig, 2015). From these results, some biological hypotheses are suggested to clarify the relationships between landscape metrics and primate exposure to the pathogen at the different scales observed.

4.1 | Increase in agent contact opportunities

Forest cover is a metric of landscape composition that affects different ecological processes of primates, such as the dispersion along the habitat, the availability of food resources, and the survival rates of individuals over time (Andrén & Andren, 1994; Fahrig, 2003, 2013; Marsh & Chapman, 2013). In this perspective, neotropical species are particularly affected by habitat loss as they depend on forests for survival (Cowlishaw & Dunbar, 2000; Johns & Skorupa, 1987). The genus *Alouatta* is known for its behavioral flexibility in the face of habitat changes and adapting to environments with different degrees of anthropogenic disturbance (i.e., surviving in small patches of habitat with a higher density of individuals) (Bicca-Marques et al., 2009, 2020; Estrada & Coates-Estrada, 1996; Estrada et al., 1999; Horwich 1998; Fortes, 2008; Fortes & Bicca-Marques, 2008; Fortes et al., 2015; Monticelli & Morais, 2015; Ribeiro & Bicca-Marques, 2005; A. S. A. Silva et al., 2017). Numerous landscapes in southern Brazil are characterized by the presence of small forest remnants surrounded by a matrix with different degrees of permeability, which could limit the movement of individuals. In many primate species, habitat loss negatively impacts the animals' ability to disperse due to fragmentation (Fahrig, 2003, 2017; Gestich et al., 2019). A small home range associated with a higher density of individuals could lead to opportunities for contact with the microorganism since greater forest cover shapes animal migrations and decreases opportunities for contact with different pathogens (Bowler & Benton, 2005; Dunning et al., 1992; Lau et al., 2010; Wiens et al., 1993).

Furthermore, the degree of isolation between patches of habitat can make it difficult for howler monkeys to disperse across the landscape (Bicca-Marques, 2003; Bicca-Marques et al., 2009; Cristóbal-Azkarate & Arroyo-Rodríguez, 2007; Pozo-Montuy & Serio-Silva, 2007; F. E. Silva & Bicca-Marques, 2013). Therefore, host movement would be one of the regulators in the transmission of the pathogen along the landscape. This helps to clarify the inverse relationship between exposure to *Leptospira* spp. and forest cover, as well as the scale of the observed effect (200 m), as explained by the lower mobility of individuals and their densification in smaller home ranges. The result in these scenarios would be a greater chance of exposure to the agent.

4.2 | Contamination by sympatric species

Howler monkeys often live in sympatry with numerous wild species, sharing habitats, tree strata, foraging areas, and food resources (Aguiar et al., 2007; Bicca-Marques et al., 2008; Geise et al., 2004; Paste & Voltolini, 2018; Teixeira et al., 2013). Many of these species can act as *Leptospira* spp. reservoirs, as is the case of marsupials and wild rodents already found that have been infected with some of the main serovars also observed in the primates of this study (Panama and Ballum; Blasdell et al., 2019; Cordeiro et al., 1981; Lins & Lopes, 1984; Santa Rosa et al., 1975). Based on the overlapping of home ranges, it is possible to assume that the contamination of plants that primates consume, through the urine of infected animals, would be one of the sources of transmission, potentiated in smaller habitats by the interaction between different species and the microorganism.

Among the elements that contribute to maintaining the transmission cycle of *Leptospira* spp. in the environment, rodents and flooded soils stand out. Rodents are important reservoirs that contribute to maintaining and transmitting bacteria in the environment. The abundance and diversity of these small mammals can be negatively affected by the type of land use and its configuration in the landscape, consequently affecting the transmission dynamics of the agent (Herrera et al., 2020). In this sense, the increase in edge density observed in our study associated with an agricultural matrix was pointed out as a negative factor for the presence of rodent communities, mainly in forest/agricultural matrix transition areas (ecotones) (Butet & Leroux, 2001; Panzacchi et al., 2010; Tõnisalu & Väli, 2022). These landscape features can cause many rodent species to move inland, reducing their presence at forest edges (Haapakoski & Ylönen, 2010). Furthermore, edges affect rodent survival due to the higher occurrence of predators and lower vegetation cover (Ferguson, 2004; Mirski & Väli, 2021; Orrock et al., 2004). A significant reduction in rodent presence in open areas has also been reported, which could help shed more light on the reduction of soil contamination by the agent in these contexts (Tõnisalu & Väli, 2022). It is interesting to note that in our study, the agricultural cover was one of the metrics measured, although it was excluded from the two analyses due to its high correlation with forest cover (VIF = 5.96 and VIF = 7.14). In the exploratory analysis of the effect of crop cover, this landscape metric was directly related to exposure to *Leptospira* spp. in the analysis without outliers ($\beta i = 0.023$; SE = 0.011; OR = 1.024; $p = 0.041$). This result corroborates the scenario mentioned above; however, the presence of rodents in the landscape fragments was not measured, and, therefore, it is a hypothesis that could be tested in these contexts.

4.3 | Decreased dispersal ability of primates

The reduced connectivity between habitats in the landscape caused by the increase in the edge density may cause a lower capacity for primates to move in a nonpermeable matrix. Given their predominantly arboreal movement, primates are particularly sensitive to disturbances that alter forest integrity and influence their home range (Bicca-Marques & Calegaro-Marques, 1995; Bolt et al., 2018; Calegaro-Marques & César bicca-Marques, 1996; Galán-Acedo, Arroyo-Rodríguez, Estrada, et al., 2019; Galán-Acedo, Arroyo-Rodríguez, Cudney-Valenzuela, et al., 2019; Oklander et al., 2010, 2017; Whitworth et al., 2016). Consequently, primate behavioral responses to habitat fragmentation would have a greater impact on exposure to *Leptospira* than habitat availability (forest cover).

The scale of the effect (1400 m) observed for this predictor in terms of exposure to the agent also reveals that landscape fragmentation can affect important ecological processes at a large scale. This perception has been corroborated by other researchers, who point to a tendency for the scale of the edge density effect to be greater in relation to forest cover, matrix permeability, and fragment density in arboreal mammals (Cudney-Valenzuela et al., 2022; Galán-Acedo et al., 2018). In this sense, we suggest that this metric of landscape configuration can operate by regulating large-scale processes, such as dispersion and metapopulation dynamics in larger territorial extensions in arboreal mammals (Cudney-Valenzuela et al., 2022; Ewers & Didham, 2006; Galán-Acedo et al., 2018).

Hence, it is possible to consider that the increase in edge density affects larger mammals, such as howler monkeys at larger spatial scales, since they can often rely on larger home ranges (Cudney-Valenzuela et al., 2022; Fahrig et al., 2019; Galán-Acedo et al., 2018; Tucker et al., 2014). Thus, in addition to the lower host mobility being an important limiting factor in the transmission and exposure to the pathogen in the habitat, the magnitude of the scale of the effect might be associated with the increase in the effects of the density of edges in the home range used by primates throughout the landscape.

4.4 | Reduction of the agent's viability in the environment

The edge effect caused by the increased fragmentation in the landscape causes changes in the biotic and abiotic conditions of the environment, including temperature, humidity, and plant composition, increasing soil exposure to sunlight and decreasing the viability of microorganisms in more compact remnants (Fortes, 2008; Laurance, 1991; Muller et al., 2010; Murcia, 1995; Tabarelli et al., 2008). Furthermore, changing temperature gradients can promote changes in ecosystems along forest edges, including increased mortality of plant species (Laurance et al., 2002). This same process also alters the availability of food resources and howler monkeys' behavioral patterns (Arroyo-Rodríguez et al., 2007; Bolt et al., 2021; Didham & Lawton, 1999; Cudney-Valenzuela et al., 2021). As a result, changes can be observed in the movement and foraging patterns of these animals, which would use the interior of the fragments with richer plant species and less use of the edges of the fragments, as reported elsewhere (Bolt et al., 2018, 2021). Thus, the increase in edge density and edge effect could negatively influence the viability of *Leptospira* spp. in the environment and, consequently, reduce the opportunities for contact with primates.

In this sense, another critical element for the risk of exposure to the agent is the soil type, a potential reservoir of pathogenic *Leptospiras* that multiply in flooded soils and can remain resting in soils with low humidity for over a year (Yanagihara et al., 2022). Thus, uncovered and dry soils in areas fragmented by the increased density of edges are unfavorable for the growth and survival of bacteria in the environment and, consequently, reduce the risk of exposure to the agent (Warnasekara et al., 2022). Therefore, the lower presence of rodents and changes in soil moisture in areas with higher border density may reduce the risk of exposure to *Leptospira* spp.

Rio Grande do Sul State has a higher incidence of human cases of leptospirosis than the Brazilian average, with a predominance in rural areas of intensive agricultural cultivation associated with irrigated crops (Barcellos et al., 2003). In this perspective, the conversion of natural areas into agricultural areas is a critical factor in landscape modification in primates environments, contributing to population decline (Chapman et al., 2006; Donald, 2004; Laurance et al., 2002). *Leptospira* spp. has been found in various rural environments in agricultural areas in several regions, and some crops may attract primates to farm areas (Lall et al., 2016). Under these conditions, a greater interface of anthropized landscapes and primate movement may influence the transmission of interspecific bacteria culminating in sharing of microorganisms (Devaux et al., 2019; Goldberg et al., 2008). Moreover, the presence of the agent is related to an intricate structure of environmental risk factors that contribute to *Leptospira* spp. exposure that is not always associated with direct contact with animals but rather contaminated soil and water (Barragan et al., 2017). As such, many gaps still need to be filled to clarify how environmental conditions favor the survival of *Leptospira* in the environment and the risk factors associated with primate exposure.

5 | CONCLUSIONS

This study indicated that native free-living nonhuman primates in Rio Grande do Sul State (southern Brazil) were previously exposed to *Leptospira* spp. in the sampled environments; moreover, decreased forest cover and edge density are landscape metrics that were associated with exposure to the agent. Understanding the presence of these serogroups/sorovars circulating in natural areas allows us to improve our knowledge of *Leptospira* spp. epidemiology and details some environmental risk factors that may contribute to their exposure. In fact, this study may encourage future investigations seeking to advance the understanding of landscape elements that contribute to the maintenance of the agent in the environment, often due to environmental modifications caused by humans. Thus, these data may assist in improving environmental surveillance and conservation strategies for neotropical primates, given the importance of the agent as a zoonotic disease and the predominance of unprotected habitats on which NHPs depend. Finally, the results of this study demonstrate how the conservation of forests benefits the protection and health of primates, considering that the loss of habitat influenced the risk of exposure to the investigated agents.

AUTHOR CONTRIBUTIONS

Elisandro O. dos Santos: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); supervision (lead); validation (lead); visualization (lead); writing—original draft (lead); writing—review and editing (lead). **Vinícius F. Klain**: Data curation (equal); formal analysis (lead); methodology (lead); validation (equal); writing—original draft (equal); Writing—review and editing (equal). **Sebastián B. Manrique**: Formal analysis (supporting); methodology (lead); validation (lead). **Rogério O. Rodrigues**: Data curation (equal); formal analysis (supporting); methodology (supporting). **Helton F. dos Santos**: Data curation (supporting); methodology (supporting); visualization (supporting). **Luís A. Sangioni**: Data curation (supporting); investigation (supporting); project administration (supporting). **Maurício G. Dasso**: Methodology (supporting). **Marco A. B. de Almeida**: Investigation (supporting); methodology (supporting); writing—review and editing (supporting). **Edmilson dos Santos**: Methodology (supporting). **Lucas C. Born**: Data curation (supporting); investigation (supporting). **José Reck**: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); writing—review and editing (supporting). **Sônia de Avila Botton**: Conceptualization (supporting); funding acquisition (lead); methodology (lead); project administration (supporting); supervision (lead); writing—review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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