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MICROBIOLOGY

Environmental factors associated with seroprevalence of *Leptospira* spp. infection in stray and shelter dogs in the Caatinga biome

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ABSTRACT: Leptospirosis is a zoonotic disease that must be studied on the One Health point of view. It is possible that there are particularities in the epidemiology of leptospirosis in Caatinga biome, where the environment is often unfavorable and challenges the adaptability of *Leptospira* spp. Overall, 100 dogs (47 shelter and 53 stray dogs) selected based on sampling calculation were used to evaluate the *Leptospira* spp. seroprevalence and associated factors. The microscopic agglutination test (MAT) was applied to detect anti-*Leptospira* spp. antibodies (cut-off titer 50). Risk factors were identified using the robust Poisson regression analysis. Twenty-four animals (24%; 95% CI = 15.6% - 32.4%) were seroreactive, antibody titers ranged from 50 to 200, and the reacting serogroups were Ballum (17%), Autumnalis (6%) and Djasiman (1%). The factors/categories associated with seropositivity were the environment where the animal stay/soil (prevalence ratio [PR] = 6.03; 95% CI for PR: 1.86 - 7.69; P < 0.001) and access to polluted water/yes (PR = 3.79; 95% CI for PR: 1.85 - 24.22; P = 0.011). The results suggested leptospirosis as a concern in the One Health context in stray and shelter dogs from the Caatinga biome despite the adverse conditions of this biome for the survive of *Leptospira* spp. on the environment. Moreover, despite being social and government issues, factors such as environment where the animal stay (soil) and access to polluted water must be carefully deemed and corrected to avoid the transmission of leptospires to animals and humans.
Key words: Leptospirosis, One Health, companion animals, epidemiology, dry climate regions.

Fatores ambientais associados com a soroprevalência de infecção por *Leptospira* spp. em cães errantes e de abrigo no bioma Caatinga

RESUMO: A leptospirose é uma doença zoonótica que deve ser estudada sob o ponto de vista da Saúde Única. É possível que existam particularidades na epidemiologia da leptospirose no bioma Caatinga, onde o ambiente é muitas vezes desfavorável e desafia a adaptabilidade de *Leptospira* spp. No total, 100 cães (47 de abrigos e 53 errantes) selecionados com base em cálculo amostral foram utilizados para avaliar a soroprevalência de *Leptospira* spp. e os fatores associados. O teste de soroaglutinação microscópica (SAM) foi aplicado para detetar anticorpos anti-*Leptospira* spp. (título 50 como ponto de corte). Os fatores de risco foram identificados utilizando a análise de regressão de Poisson robusta. Vinte e quatro animais (24%; IC 95% = 15,6% - 32,4%) foram soropositivos, os títulos de anticorpos variaram de 50 a 200, e os serogrupos reagentes foram Ballum (17%), Autumnalis (6%) e Djasiman (1%). Os fatores/categorias associados à soropositividade foram o ambiente onde o animal se encontra/solo (razão de prevalência [RP] = 6,03; IC 95% para RP: 1,86 - 7,69; P < 0,001) e o acesso a água poluída/sim (RP = 3,79; IC 95% para RP: 1,85 - 24,22; P = 0,011). Os resultados sugerem a leptospirose como uma preocupação no contexto da Saúde Única em cães errantes e de abrigos no bioma Caatinga, apesar das condições adversas desse bioma para a sobrevivência de *Leptospira* spp. no ambiente. Além disso, apesar de serem questões sociais e governamentais, fatores como o ambiente onde o animal fica (solo) e o acesso à água poluída devem ser cuidadosamente analisados e corrigidos para evitar a transmissão de leptospiras para animais e humanos.
Key words: Leptospirose, Saúde Única, animais de estimação, epidemiologia, regiões de clima quente.

Leptospirosis is an infectious bacterial zoonotic disease that can infect domestic and wild animals, and can be accidentally transmitted to humans. Generally, in endemic areas, dogs shed a low amount of leptospires and can act as sentinels for human risk of exposure. In some cases, dogs can be asymptomatic and can shed leptospires, especially if they are chronically infected with the serogroup Canicola, but this is not the most common frame (SANT'ANNA et al., 2017; SYKES et al., 2023). In Brazil, according to the Ministry of Health, 2,711 confirmed cases of human leptospirosis were reported in 2023 (data updated on 14 December 2023), of which 236 (lethality of 8.7%) died (BRASIL, 2023).

The disease is caused by species of *Leptospira* spp. and currently, based on phylogenetic analyses, the genus is divided into three lineages that constitute the level of pathogenicity: saprophytic (26 species), intermediate (21 species) and pathogenic (17 species). Intermediate species share a nearly

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common ancestor with pathogenic species; although, they exhibit moderate pathogenicity in humans and animals (VINCENT et al., 2019). In dogs, especially stray dogs, infection occurs mainly through exposure to water contaminated with urine or tissues from infected animals, especially in scenarios where there are high levels of rainfall and in areas where the soil has a neutral or slightly alkaline reaction (ALTHEIMER et al., 2020). Various occupations can predispose to contact with the bacteria, such as veterinarians, fishermen, farmers and cleaners in unsanitary areas (ADLER & DE LA PEÑA-MOCTEZUMA, 2010). The prevalence of leptospirosis in dog population has been estimated in Brazil as being 19.7%, and Canicola, Icterohaemorrhagiae and Autumnalis have been the most frequently found serogroups (ESTEVES et al., 2023).

The Caatinga biome occurs only in Brazil and has characteristics of dry forests, high temperatures and low humidity, as well as broad biodiversity. The average temperatures are28 ºC, reaching 40 ºC and precipitation that varies from 400 to 800 mm annually and a rainy period that lasts three to four months, generating negative water balance which, combined with high solar radiation and high evaporation rates, contribute to a significant loss of water available in the soil (ZANELLA, 2014). It covers an area of 826,411 km² (11% of the national territory). It is present in all states of the Northeast region of Brazil, as well as part of the north of Minas Gerais. It has specific vegetation, which makes it unique to the region and therefore offers epidemiological conditions that should be assessed differently from other regions of Brazil and the world. It is possible that there are particularities in the epidemiology of leptospirosis in dry climate regions, where the environment is often unfavorable and challenges the adaptability of *Leptospira* spp. forcing the agent to seek alternative routes of transmission (NOGUEIRA et al., 2020), which is probably strictly linked to reservoir species. Considering the great impact on public health of *Leptospira* spp. infection, it must be studied from the point of view of the One Health initiative (SANTOS et al., 2022). In this context, this surveyevaluated the seroprevalence of stray and shelter dogs in the Caatinga biome, and to identify factors associated with the seroprevalence.

The research was carried out in Patos county, state of Paraíba. The municipality is a commercial centre and has 108,766 inhabitants, 96.63% located in urban areas and 3.37% in rural areas. The climate is hot and dry, with average annual temperatures of 35º in summer and 32º in winter and annual rainfall of approximately 700 mm, the historical average, as well as being included in the Caatinga Biome.

The minimum number of animals to be selected was determined using the formula for simple random sampling (THRUSFIELD & CHRISTLEY, 2018), considering the following parameters: estimated prevalence of 50% (sample maximisation), 95% confidence level (Z = 1.96) and sampling error of 10%, which resulted in a minimum sample size of 96 dogs. However, 100 dogs over three months old of both sexes and different breeds were used. Overall, 47 shelter dogs collected by the Patos city kennel in partnership with the NGO Adota Patos, and 53 stray dogs from the surroundings of the Veterinary Hospital, Universidade Federal de Campina Grande (UFCG), in Patos-PB, were assessed. Blood was collected by puncturing the cephalic vein using 3 mL disposable syringes with a 25x0.7 mm needle between April and June 2023. At the Laboratory of Communicable Diseases/UFCG, the samples were centrifuged at 1,512 g for 10 min and the serum samples were stored at -20 ºC until *Leptospira* spp. serology was performed.

At the moment of blood collection, a epidemiological questionary was filled with information on the sex of the animal (male, female), accsess to polluted water (no, yes), contact with other dogs (no, yes), condition of housing (stray, shelter), environment where the animal stay (cement, soil, cement/soil) and age (young [< 2 years], adult [≥ 2 years]).

The microscopic agglutination technique (MAT) was applied with a collection of live antigens composed of 24 serovars belonging to 17 pathogenic serogroups of five species from the Pasteur Institute, France, and provided by the Universidade Federal Fluminense (UFF): *L. interrogans* serovars Copenhageni, Canicola, Autumnalis, Wolffi, Hardjoprajitno, Icterohaemorrhagiae, Pomona, Kennewicki, Hebdomadis, Pyrogenes, Bratislava and Australis; *L. santarosai* serovars Guaricura, Shermani and Canalzoni; *L. borgpetersenii* serovars Javanica, Tarassovi, Ballum, Mini and Castellonis; *L. kirschneri* serovars Grippotyphosa and Cynopteri; *L. noguchi* serovars Panama and Louisiana (WOAH, 2021). Sera were screened at a 1:50 dilution, and antibody titers were determined as the highest dilution with at least 50% of agglutinated leptospires, and animals were deemed positive when they presented reciprocal titers ≥ 50.

To conduct the analysis of factors associated with seropositivity, bivariate analysis was initially performed, in which each independent variable underwent an association analysis in relation to the dependent variable (seropositivity in serological test). Variables with P-value ≤ 0.2 in the Chi-square test or G test were selected for multivariable analysis using robust Poisson regression. Collinearity between independent variables

was verified by a correlation analysis; for those variables with a strong collinearity (correlation coefficient >0.9), one of the two variables was excluded from the multiple analysis according to the biological plausibility (DOHOO et al., 2003). The significance of the model was verified with Omnibus test, the significance level adopted in the multiple analysis was 5%, and the software used was SPSS for Windows version 20.0.

Of the 100 dogs analysed, 24 animals (24%; 95% CI = 15.6% - 32.4%) were seroreactive at 1:50 cut-off, and three (3%; 95% CI = 0.6% - 8.5%) at 1:100 cut-off. Antibody titers ranged from 50 to 200, and the reacting serogroups were Ballum (17%), Autumnalis (6%) and Djasiman (1%) at 1:50 cut-off, and Autumnalis (2%) and Ballum (1%) at 1:100 cut-off (Table 1). Table 2 shows the results of the bivariate analysis, and the variables selected for the robust Poisson regression were: access to polluted water ($P < 0.001$), condition of housing ($P = 0.192$), environment where the animal stay ($P = 0.007$) and age ($P = 0.117$). In the final model, the factors/categories associated with seropositivity were the environment where the animal stay/soil (prevalence ratio [PR] = 6.03; 95% CI for PR: 1.86 - 7.69; $P < 0.001$) and access to polluted water/yes (PR = 3.79; 95% CI for PR: 1.85 - 24.22; $P = 0.011$). The final regression model showed statistical significance (Omnibus test: Chi-squared = 22.6; degrees of freedom = 4; $P < 0.001$).

The fieldwork presented the main limitation of collecting a large amount of data to used in the risk factor analysis. Indeed, the collection of epidemiological information from stray and shelter dogs is very tricky due to the characteristics of the populations. However, the data collected were carefully checked to avoid biases.

Despite the unfavorable conditions of the Caatinga biome for the survive of *Leptospira* spp. on the environment and, therefore, decreasing possibility of exposure to leptospires, the seroprevalence found was high and reinforce the exposure of animals to *Leptospira* spp. Indeed, in recent surveys with livestock of the caatinga Biome the seropositivities (ewes, 13.33%; rams, 12.5%) were lower than that reported in this study; nevertheless, there were high PCR-positivity rates mainly in tissues and fluids of the genital tract (SANTOS et al., 2022; SOARES et al., 2021), suggesting that the genital route is important for leptospire transmission in semiarid conditions. However, this condition is more frequent for some serovars/serogroups, such as Hardjo for cattle (LOUREIRO & LILENBAUM, 2020), and probably the role of chronic renal shedders is more important for some other serovars/serogroups, such as Canicola for dogs (SYKES et al., 2023).

Table 1 - Serogroups and respective antibody titers to *Leptospira* spp. in 100 stray/shelter dogs in the Caatinga biome.

Serogroup	--------Antibody titer--------			Total (%)
	50	100	200	
Ballum	16	1	0	17 (17)
Autumnalis	4	1	1	6 (6)
Djasiman	1	0	0	1 (1)
Total (%)	21 (21)	2 (2)	1(1)	24 (24)

Usually, the cut-off point for serology is titer 100, but in this survey the cut-off 50 was used. The efficiency of the 50 cut-off point in serology had already been suggested in ewes (NOGUEIRA et al., 2020), and in rams (SOARES et al., 2021) in semiarid conditions with more efficient results as the highest sensitivities for MAT were obtained with this cut-off compared to 100, and, regardless of the material used in PCR, the highest sensitivities were obtained considering this cut-off point, i.e., fewer false negative results were obtained. The possibility of detecting an infected animal increases when a protocol is adapted to the region studied (SOARES et al., 2021). This is very important from an epidemiological and infection control point of view, as it avoids maintenance of infected animals, since *Leptospira* spp. subclinical infections are very common (SANTOS et al., 2022) depending on the animal species and on the *Leptospira* spp. serovar (ADLER & DE LA PEÑA-MOCTEZUMA, 2010).

The Ballum serogroup, the most frequent in this study; although, less common in surveys carried out in Brazil, was the most frequent in sheep from different slaughterhouses in the state of Paraíba (COSTA et al., 2016). Its main reservoirs are small domestic mice or rats (BHARTI et al., 2003), which intermittently eliminate leptospires through urine. The presence of Autumnalis serogroup in our research must be highlighted. Serorectivity of this serogroup is very common in small ruminants, reinforcing the condition of these animals are frequently exposed to it. It is worth noting that Autumnalis is not included in vaccines sold in Brazil, which further reinforces the importance of isolating this serovar and evaluating the need of its inclusion on new vaccines against leptospirosis (COSTA et al., 2016; NOGUEIRA et al., 2020). Furthermore, the reservoirs of this serogroup are rodents (FAINE et al., 1999).

The epidemiology of leptospirosis is complex, with a broad diversity of hosts and reservoirs, different *Leptospira* spp. species and a

Table 2 - Bivariate analysis for factors associated with *Leptospira* spp. seroprevalence in stray and shelter dogs from Caatinga biome.

Variable	Categories	Total No. of animals	No. of positive animals (%)	P-value
Sex	Male	48	11 (23)	0.993
	Female	52	13 (25)	
Accsess to polluted water	No	63	7 (11.1)	< 0.001*
	Yes	37	17 (45.9)	
Contact with other dogs	No	25	5 (20)	0.787
	Yes	75	19 (25.3)	
Condition of housing	Stray	53	16 (30.2)	0.192*
	Shelter	47	8 (17)	
Environment where the animal stay	Cement	44	18 (40.9)	0.007*
	Soil	20	4 (20)	
	Cement/soil	26	2 (7.7)	
Age	Young	77	18 (23.4)	0.117*
	Adult	23	6 (26.1)	

strong environmental component, so that the infection must be studied fom the One Health point of view. Two enviromental variables were associated with increasing seroprevalence in dogs: environment where the animal stay (soil) and access to polluted water. It's very well-known that animals carrying leptospires eliminate the bacteria through urine and contaminate the environment, and humans and animals can get infected through direct or indirect contact with contaminated water or soil (ADLER & DE LA PEÑA-MOCTEZUMA, 2010). Recently, in Nicaragua, *Leptospira* spp. was isolated in 20 (47.6%) of 42 soil samples (FLORES et al., 2020). The access to polluted water was also a factor associated with seroprevalence. This factor has been reported by other authors (HERNÁNDEZ RAMÍREZ et al., 2017; MURPHY, 2018). Moreover, the exposure to water and soil contaminated by the urine of infected animals is the most common route of transmission to humans and domestic animals (SCHNEIDER et al., 2015; MIOTTO et al., 2018).

Therefore, the results obtained suggested leptospirosis as a concern in the One Health context in stray and shelter dogs from the Caatinga biome despite the unfavorable conditions of this biome for the survive of *Leptospira* spp. on the environment. In addition, despite being social and government issues, factors such as environment where the animal stay (soil) and access to polluted water must be carefully deemed and corrected to avoid the transmission of leptospires to animals and humans.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Maylson Andrade Severo, Clécio Limeira Henrique, Sabrinna de Sales Araújo, Roseane de Araújo Portela, Nathália Mariade Andrade Magalhães, Karla Nayalle de Souza Rocha, Clebert José Alves, Carolina de Sousa Américo Batista Santos and Sérgio Santos de Azevedo contributed equally to conceptualization, writing-original draft, and writing-review and editing

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Animal Ethics Committee (CEUA/CSTR/UFCG) under protocol no. 13/2022.

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