Research Square

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Isolation of pathogenic Leptospira in drinking water cisterns in the Caatinga biome

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Abstract

The Caatinga biome is found exclusively in Brazil, covering approximately 10.1% of the national territory, and is characterized by a semiarid climate, high biodiversity, and pronounced climatic seasonality, with approximately three months of rainfall followed by nine months of drought. In this context, supply cisterns represent critical convergence points at the human–animal–environment interface, where shared access to water resources may facilitate the circulation of infectious agents. This study aimed to investigate the occurrence of *Leptospira* spp. in cistern used for human consumption in the Paraíba state, Caatinga biome, semiarid of Brazil. Ninety-six cistern water samples were cultured in EMJH medium and screened by conventional polymerase chain reaction (PCR) targeting the *16S rRNA* gene. Positive samples were further tested by PCR targeting *LipL32* to detect pathogenic leptospires. The *LipL32*-positive isolates were characterized by partial *secY* gene sequencing and phylogenetic analysis. *Leptospira* spp. were detected in 19 of 96 samples (19.79%; 95% CI: 12.40–29.24%). Six samples were positive for *LipL32*, corresponding to 31.58% of *Leptospira*-positive samples and 6.25% (95% CI: 2.32–13.09%) of all samples. Neither *Leptospira* spp. nor pathogenic *Leptospira* detection was significantly associated with the water source. Partial *secY* gene sequencing of four pathogenic isolates identified one as *Leptospira interrogans* with 100% sequence identity; the other three were classified as *L. santarosai*, achieving 91% sequence identity. These findings highlight the importance of microbiological monitoring of water stored in alternative supply systems and reinforce environmental surveillance within a One Health framework in the Brazilian semi-arid region.

1 Introduction

Leptospirosis is a neglected zoonosis with worldwide distribution caused by pathogenic species of *Leptospira* spp. It affects human populations in both rural and urban areas and is endemic mainly in countries with subtropical and humid tropical climates. In addition to its impact on public health, the disease causes significant economic losses in animal production, mainly due to reproductive disorders. In humans, approximately 1 million cases and 60,000 deaths are estimated to occur annually; however, the exact number of human cases remains unknown due to underdiagnosis or misdiagnosis, with outbreaks commonly associated with floods and hurricanes [1–3].

The Caatinga biome, the only exclusively Brazilian biome, occupies approximately 862,640 km², encompassing practically the entire Northeast region and the northern part of Minas Gerais state. It is a well-defined phytogeographical unit located in a semiarid climate region and is recognized as one of the largest and most densely populated dryland areas on the planet. However, despite its ecological relevance and significant biodiversity, the Caatinga remains one of the less investigated Brazilian biomes from a scientific perspective [4].

Rainwater harvesting is a fundamental strategy that allows families to remain in semiarid regions, such as the Paraíba hinterland. The One Million Rural Cisterns Program (P1MC), managed since 2001 by the Articulation of the Brazilian Semi-Arid Region (ASA Brasil), a network of civil society organizations,

promotes the construction of cement-slab cisterns next to rural residences to collect water through gutters installed on rooftops. Each cistern has a capacity of 16,000 L, a volume sufficient to meet the basic domestic water needs, particularly for drinking and cooking, of families of up to six people during droughts. The program also provides beneficiaries with training on cistern maintenance to ensure the quality of the water stored in the reservoir [5].

Although the hot and dry climatic conditions of the Brazilian semiarid region are considered unfavorable to the environmental survival of *Leptospira* spp., studies conducted in the Caatinga biome have demonstrated the circulation of this pathogen. High frequencies of *Leptospira* spp. have been detected in both domestic [6–8] and wild animals [9–11], indicating the persistence of the agent in this ecosystem. Even so, knowledge about the survival of leptospires in the environment, particularly in aquatic environments, remains limited. This gap restricts the understanding of the epidemiology of the disease and reinforces the need for studies aimed at improving its prevention and control.

Despite the epidemiological relevance of leptospirosis and the documented circulation of *Leptospira* spp. in domestic and wild animals in the Caatinga biome, studies investigating the presence of these pathogens in water sources used for human supply in the Brazilian semiarid region remain scarce. Considering the importance of water as a potential reservoir and transmission route for infectious agents, particularly in contexts of water vulnerability, this study aimed to investigate the occurrence of *Leptospira* spp. in water samples from cisterns used for human consumption in the Caatinga biome.

2 Methods

In July and August 2025, a cross-sectional study was conducted in the rural areas of the municipalities of Aparecida, São José da Lagoa Tapada, and Sousa, in the hinterland of the state of Paraíba, Brazil, covered by the Caatinga biome (Table 1). According to Brazilian Institute of Geography and Statistics (IBGE) [12–14], the municipalities have a combined population of 82,345 inhabitants.

Table 1
Demographic characteristics and number of cisterns in the municipalities of Paraíba included in the study

Municipality	**Number of inhabitants**	**Population density (inhab./km^2)**	**Number of cisterns**
Aparecida	7,960	27.31	781
São José da Lagoa Tapada	7,126	21.35	1,135
Sousa	67,259	92.33	2,212
TOTAL	**82,345**	**—**	**4,128**
Source: IBGE [12–14]			

The sample size was estimated considering simple random sampling, adopting a 95% confidence level, an expected prevalence of 50%, and a sampling error of 10% [15]. A prevalence of 50% was used because no previous studies were available to estimate the occurrence of *Leptospira* spp. in cistern water intended for human consumption in the Caatinga biome; this represents a conservative approach to sample size calculation. Cisterns were selected by convenience sampling, considering the accessibility of the properties and the families' agreement to participate in the study.

Ninety-six water samples were collected from cistern-type reservoirs. Samples were collected in sterile 15 mL tubes. From each sample, a 1 mL aliquot was inoculated into a single tube containing 5 mL of EMJH liquid culture medium (Difco, Detroit, MI, USA), supplemented with 40 µg/mL sulfamethoxazole, 20 µg/mL trimethoprim, 5 µg/mL amphotericin B, 400 µg/mL fosfomycin, and 100 µg/mL 5-fluorouracil. The samples were transported in insulated containers to the laboratory and incubated at 28°C for 24 h. After 24 h, subcultures were performed in EMJH medium (liquid and semi-solid) supplemented with 5-fluorouracil and maintained at 28°C. Bacterial growth was monitored weekly for 16 weeks using dark-field microscopy to identify the characteristic morphology of *Leptospira* spp. Subcultures were performed every two weeks, and a sample was deemed positive when growth was observed in at least one of them. Samples showing no evidence of growth after 16 weeks were considered negative and discarded. Cultures with morphology suggestive of *Leptospira* spp. were subjected to molecular identification.

Bacterial DNA was extracted from the cultures using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA), strictly following the manufacturer's instructions. For the detection of *Leptospira* spp., amplification of a 330 bp fragment of the *16S rRNA* (*rrs*) gene was performed using primers Lep1 (5'-GGCGGCGCGTCTTAAACATG-3') and Lep2 (5'-TTCCCCCCATTGAGCAAGATT-3') [16]. Positive samples from this initial screening were subjected to a second PCR for pathogenicity differentiation, using primers *LipL*32-45F (5'-AAG CAT TAC CGC TTG TGG TG-3') and *LipL*32-286R (5'-GAA CTC CCA TTT CAG CGA TT-3'). These primers amplify a 242 bp fragment of the *LipL*32 gene, which encodes an outer membrane lipoprotein exclusive to pathogenic species [17].

Samples positive for the *LipL*32 gene were subjected to amplification and bidirectional Sanger sequencing of a 549-bp fragment of the *secY* gene using specific primers [18]. Consensus sequences were generated in MEGA v12 [19] and compared with GenBank sequences using BLASTn. Phylogenetic analysis was carried out using MUSCLE alignment [20] and the Maximum Likelihood method under the Kimura 2-parameter model with gamma-distributed rate variation (K2 + G), with 1,000 bootstrap replicates and partial deletion (95% site coverage), yielding 216 aligned positions. *Leptospira licerasiae* (OR112585.1) was used as the outgroup. The sequences generated were deposited in GenBank under accession numbers PZ723147–PZ723150.

Absolute and relative frequencies were calculated using descriptive statistics. Data were tabulated in Microsoft Excel® and analyzed using IBM SPSS Statistics version 20.0. The association between

Leptospira spp. positivity and the water source used was evaluated using Fisher's exact test (Freeman-Halton extension for R x C tables), adopting a significance level of 5% ($p < 0.05$).

3 Results

Of the 96 water samples analyzed, 19 showed amplification of the *Leptospira 16S rRNA* gene fragment by conventional PCR using the Lep1 and Lep2 primers, corresponding to a prevalence of 19.79% (95% CI: 12.40–29.24%) (Table 2). Among the 19 samples positive for *Leptospira* spp., six were positive by PCR targeting the *LipL32* gene, corresponding to 31.58% of the samples positive for *Leptospira* spp. Considering the total number of samples analyzed (n = 96), the frequency of pathogenic *Leptospira* was 6.25% (95% CI: 2.32–13.09%). Regarding the type of water supply used to fill the cisterns, the prevalence of *Leptospira* spp. ranged from 0% (rain and pond) to 21.43% (water truck), whereas the prevalence of pathogenic *Leptospira* ranged from 0% (pond, rain/water truck, and rain/pond) to 14.3% (water truck). No significant differences were observed among water sources for either *Leptospira* spp. ($p = 0.934$) or pathogenic *Leptospira* ($p = 0.792$).

Table 2
Prevalence of *Leptospira* spp. and pathogenic *Leptospira* according to the water source of the cisterns.

Water source	**Number of samples**	**Samples positive for *Leptospira* spp.**	**Frequency of samples positive for *Leptospira* spp.**	**Samples positive for pathogenic *Leptospira***	**Frequency of samples positive for pathogenic *Leptospira***
Rain	66	14	21.21%	4	6.1%
Water truck	14	3	21.43%	2	14.3%
Pond	6	1	16.67%	0	0%
Rain and water truck	9	1	11.11%	0	0%
Rainfall and pond	1	0	0%	0	0%
TOTAL	**96**	**19**	**19.79%**	**6**	**6.25%**

Of the six samples positive for *LipL32*, four yielded amplification products suitable for the subsequent partial sequencing of the *secY* gene, whereas the remaining two did not yield satisfactory amplification for this analysis. After sequence editing and generation of the consensus sequences, fragments of 444, 409, 357, and 296 bp, corresponding to samples Lepto 53, Lepto 78, Lepto 79, and Lepto 71, respectively (GenBank accession numbers PZ723147–PZ723150), were analyzed. Phylogenetic analysis (Fig. 1) revealed that the four *Leptospira* samples were distributed across two distinct lineages. Samples Lepto 53, Lepto 78, and Lepto 79 clustered into a well-supported clade (bootstrap = 99%) with the *Leptospira* sp. reference sequence OK345245.1. This grouping was positioned within a broader lineage containing

reference sequences of *L. santarosai*, with 91% bootstrap support, indicating a close phylogenetic relationship between these three samples and *L. santarosai*. In contrast, sample Lepto 71 clustered within the *L. interrogans* lineage, showing a close phylogenetic relationship with the reference sequence CP186581.1, with 100% bootstrap support. The clade containing the *L. interrogans* sequences showed 76% support and was phylogenetically distinct from the clade containing Lepto 53, Lepto 78, and Lepto 79. Taken together, these results indicate the presence of at least two phylogenetically distinct *Leptospira* lineages among the analyzed samples, one associated with *L. santarosai* (Lepto 53, 78, and 79) and the other with *L. interrogans* (Lepto 71).

4 Discussion

The detection of *Leptospira* spp. in 19.79% of drinking water samples, including the identification of pathogenic species in 6.25% of the samples, demonstrates the environmental occurrence of the agent in reservoirs used by humans in the Caatinga biome. These findings have important epidemiological significance, as leptospirosis remains one of the bacterial zoonoses with the greatest global impact, being associated with high morbidity and mortality and frequently linked to exposure to contaminated water [1, 2]. The environmental persistence of *Leptospira* plays a central role in maintaining the transmission chain [21], particularly in areas where populations depend on alternative water sources.

Despite the widespread use of cisterns as a water supply strategy in semiarid regions, studies specifically investigating the occurrence of *Leptospira* in stored water intended for human consumption remain scarce. Most research focuses on surface water, soil, and other environmental reservoirs, predominantly using molecular methods to detect the agent [1]. This gap makes direct comparisons with the findings of the present study difficult, but it also highlights its originality by demonstrating the occurrence of *Leptospira* spp. and the isolation of viable pathogenic leptospires from cistern water in the Caatinga biome. These findings expand current knowledge of the environmental epidemiology of leptospirosis in reservoirs used for human consumption in the Brazilian semiarid region.

The detection of *Leptospira* spp. in cisterns supplied by rainwater deserves attention, considering that these systems represent one of the main strategies for accessing water for human consumption in the Brazilian semiarid region [5, 22], with positive impact on population health. Data from the Institute for Applied Economic Research (Ipea) associate the implementation of the P1MC with a reduction in infant mortality from acute diarrheal diseases (ADD), with decreases of up to 69% in municipalities where the program had been in operation for longer periods, highlighting its relevance to health promotion and water security [23].

However, increased access to water does not, in itself, guarantee its microbiological quality. Studies have shown that cistern water may present significant microbiological contamination when adequate sanitary protection measures are not adopted, as the safety of stored water depends on collection conditions, the cleanliness of roofs and gutters, reservoir protection, and management practices during storage [24, 25]. Studies conducted in the state of Rio Grande do Norte, Brazil, have identified *E. coli*

contamination in cistern water [24]. Although the P1MC has promoted important advances in water security in the semiarid region, the detection of *Leptospira* reinforces the importance of continuously monitoring its microbiological quality.

The wide diversity of maintenance hosts described in the Brazilian semiarid region demonstrates that the environmental circulation of *Leptospira* results from interactions among domestic animals [6–8, 26, 27], wildlife [9–11], and the environment, contributing to the maintenance of the agent at the interface environment-animals-humans. Synanthropic rodents constitute the main reservoirs of pathogenic species [3], shedding viable leptospires in their urine for prolonged periods and promoting environmental contamination. In this context, water contamination can occur on roof surfaces, in gutters, during rainwater runoff, or even in the vicinity of homes, particularly on rural properties where there is intense interaction between domestic animals and wildlife. This scenario is consistent with the findings of the present study, reinforcing the possibility that cistern water may act as a link between these animal reservoirs and potential human exposure.

The finding of pathogenic *Leptospira* in water distributed by water trucks increases the epidemiological relevance of the study and is particularly concerning, considering that Brazilian legislation establishes requirements to ensure the quality of water for human consumption. Operation Water Truck is regulated by Interministerial Decree No. 1, of July 25, 2012 [28], while GM/MS Decree No. 888, of May 4, 2021 [29] establishes procedures for controlling and monitoring the quality of water for human consumption, including water distributed by transport vehicles. Therefore, the detection of pathogenic *Leptospira* suggests the possibility of failures at some stage of the supply chain, such as collection, treatment, transport, or domestic storage, reinforcing the need to monitor the quality of water distributed to rural populations.

PCR is widely used in environmental studies of *Leptospira* because it allows the detection of the agent even in samples with low bacterial loads or in which microbiological isolation is hindered by the presence of competing microorganisms [30, 31]. In this study, confirmation of the isolates through amplification of the *LipL32* gene demonstrates the relevance of the findings, since the isolation of pathogenic *Leptospira* from environmental samples is considered rare. In a study of water and soil samples [30], 98% of the isolated leptospires were identified as belonging to the S1 clade, which is composed predominantly of saprophytic leptospires. Similar finding was reported in other studies [1, 30–33] demonstrating the predominance of saprophytic leptospires in environmental samples.

The identification of *L. interrogans* represents one of the main findings of this study, confirming that the observed environmental occurrence is not restricted to saprophytic leptospires but also involves a species of recognized public health importance. In the Caatinga biome, the molecular identification of this species by sequencing was recently described in goats raised under field conditions [27]. Together, these findings expand current knowledge of the ecology of *Leptospira* in the Brazilian semiarid region and indicate that water reservoirs used by rural populations may favor the environmental persistence of the bacterium.

Three isolates were classified as *Leptospira* sp., as sequencing and phylogenetic analyses did not allow their reliable attribution to a previously described species. Although they showed phylogenetic proximity to *L. santarosai*, a species previously detected in environmental samples [30], the samples formed a distinct clade from the reference sequences of this species, suggesting a related environmental lineage or a taxon that remains insufficiently characterized. However, this hypothesis should be interpreted with caution, as the partial *secY* gene fragment, together with the different lengths of the sequences analyzed, limits taxonomic resolution. Thus, the conservative classification of these isolates as *Leptospira* sp. represents the most appropriate approach, and studies using additional molecular markers or genomic sequencing are needed to clarify their taxonomic position.

The isolation of pathogenic *Leptospira* from samples collected in the Caatinga biome constitutes, to the best of our knowledge, the first evidence reported in the literature of a viable isolate of this bacterial group being obtained from an aquatic environment in this ecosystem. This finding improves knowledge of the ecology of *Leptospira* spp. in the Caatinga, a biome frequently characterized by environmental conditions deemed unfavorable to the prolonged persistence of the agent due to high temperatures and limited water availability [3, 21]. Although these environmental characteristics may limit bacterial survival, conditions within cisterns, such as protection from direct solar radiation and the prolonged retention of stored water, may favor the persistence of the agent, as suggested for other aquatic environments [1, 34].

From the perspective of food and nutritional security, the results also have relevant implications. Although initiatives aimed at rainwater harvesting and storage have significantly contributed to improving living conditions in the Brazilian semiarid region [22, 23], persistent socioeconomic vulnerabilities, coupled with limitations in sanitation infrastructure and inadequate management of water storage systems, may favor continued exposure of the population to waterborne pathogens [24, 25], reinforcing the need for integrated actions in environmental surveillance and health education.

Among the study's limitations, the small number of samples in some water supply categories and the cross-sectional design, which limits causal inferences, should be highlighted. On the other hand, the combination of microbiological isolation, PCR, and molecular characterization strengthens the reliability of the findings and represents a methodological advantage compared with most environmental studies, which frequently rely exclusively on molecular techniques.

The results highlight the importance of the One Health approach for understanding the epidemiology of leptospirosis in the Brazilian semiarid region [35]. The circulation of *Leptospira* among domestic animals, wildlife, and the environment, together with the detection of the agent in drinking water, reveals a complex epidemiological cycle that transcends isolated ecological compartments. Thus, the findings of this study provide evidence that can support environmental surveillance strategies and inform the improvement of public policies aimed at protecting the health of populations in the semiarid region, reducing the risk of leptospirosis transmission, and advancing knowledge of the ecology of *Leptospira* in the Caatinga biome.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

CRAF: Conceptualization, Investigation/Methodology, Writing—original, Formal analysis. GMSF: Investigation/Methodology. RBSS: Investigation/Methodology. FRBN: Project administration/Resources. TFF: Investigation/Methodology, Writing—review & editing. VLRV: Investigation/Methodology, Writing—review & editing. CSABS: Project administration/Resources. CJA: Project administration/Resources. SSA: Conceptualization, Supervision, Software, Funding, Writing—review & editing.

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Data Availability

The nucleotide sequences generated in this study have been deposited in GenBank under accession numbers PZ723147–PZ723150 (https://www.ncbi.nlm.nih.gov/nuccore/PZ723147; https://www.ncbi.nlm.nih.gov/nuccore/PZ723148; https://www.ncbi.nlm.nih.gov/nuccore/PZ723149; https://www.ncbi.nlm.nih.gov/nuccore/PZ723150). The remaining data supporting the findings of this study, including the results for each of the 96 water samples, are available from the corresponding author upon reasonable request.

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Figures

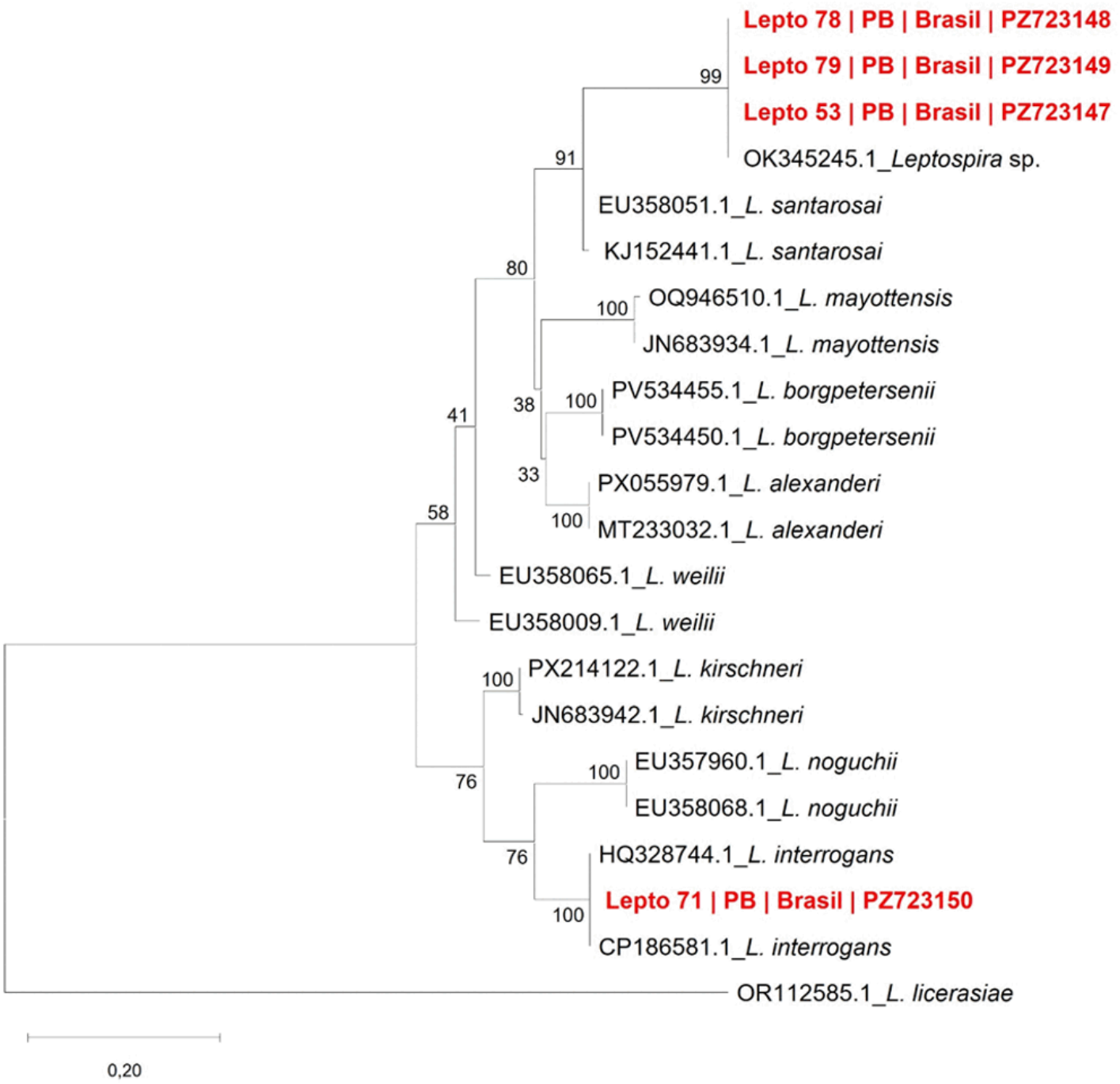

Figure 1

Phylogenetic tree based on partial sequences of the *secY* gene from species of the genus *Leptospira*, with bootstrap values (%) indicated at the nodes. Isolates from this study are shown in red (Lepto 53, 71, 78, and 79 | Paraíba, Brazil). *Leptospira licerasiae* (OR112585.1) was used as an outgroup. Scale bar: 0.20 substitutions per site