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Bovine genital leptospirosis: Findings in bulls maintained in Caatinga biome conditions[1]

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ABSTRACT.- Barnabé N.N.C., Soares R.R., Nogueira D.B., Araújo Júnior J.P., Malossi C.D., Costa D.F., Silva M.L.C.R., Higino S.S.S., Azevedo S.S. & Alves C.J. 2023. **Bovine genital leptospirosis: Findings in bulls maintained in Caatinga biome conditions**. *Pesquisa Veterinária Brasileira 43:e07376, 2023*. Unidade Acadêmica de Medicina Veterinária, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Av. Universitária 110, Patos, PB 58708-110, Brazil. E-mail: sergio.santos@professor.ufcg.edu.br

Leptospirosis is a disease that causes economic and social impact, as it affects wild and domestic animals and humans. There may be peculiarities in the epidemiology of this disease in the Caatinga biome, Brazil, where the environment is adverse and the etiologic agent, *Leptospira* spp., requires alternative transmission routes. Considering that in bovine leptospirosis the genital carrier is constantly neglected and the lack of reports on the role of bulls in the epidemiology of the bovine genital leptospirosis (BGL) syndrome, mainly in semiarid conditions such as Caatinga biome, this study aimed to investigate bulls maintained in Caatinga biome conditions as genital carriers of leptospires. Urinary tract (urine, bladder, and kidney) and reproductive tract (vas deferens, cauda epididymis, and vesicular gland) samples were collected from 42 slaughtered bulls. Microscopic agglutination test (MAT), polymerase chain reaction (PCR), and microbiological isolation were included as diagnostic methods. Anti-*Leptospira* spp. antibodies were found in 17 (40.48%) animals, while 26 animals (61.90%) had at least one organ or urine with leptospiral DNA, and 10 animals (23.81%) were positive at bacteriological culture. Sequenced samples targeting the *LipL*32 gene showed 99% similarity with *Leptospira borgpetersenii*. Molecular analysis of samples from the vas deferens and cauda epididymis is recommended for the diagnosis of genital leptospirosis in bulls and, if it is impossible to collect these tissues, semen can be used. In conclusion, this study provides important information relating to bulls from the Caatinga biome, Brazil, as carriers of genital leptospirosis. The results indicate that, even under adverse environmental conditions, leptospires may survive and propagate, mainly due to the characteristic of genital carriers for the sexually spreading of adapted *Leptospira* species without influence by external variables. Thus, prevention and control strategies for bovine leptospirosis need to include actions aimed at the genital carrier.

INDEXING TERMS: *Leptospira* spp., semiarid, PCR, bacteriological culture, genital carrier, bulls, Caatinga.

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RESUMO.- [**Leptospirose genital bovina: achados em touros mantidos em condições do bioma Caatinga.**] A leptospirose é uma doença que causa impacto econômico e social, pois afeta animais silvestres, domésticos e humanos. É possível que existam peculiaridades na epidemiologia desta doença no bioma Caatinga, Brasil, onde o ambiente é adverso e o agente etiológico, *Leptospira* spp., requer vias alternativas de transmissão. Considerando que na leptospirose bovina o portador genital é constantemente negligenciado e a falta de relatos sobre o papel dos touros na epidemiologia da síndrome da leptospirose genital bovina (BGL), principalmente em condições semiáridas como no bioma Caatinga, este estudo teve como objetivo investigar touros mantidos em condições do bioma Caatinga como portadores genitais de leptospiras. Amostras do trato urinário (urina, bexiga e rim) e do trato reprodutivo (ducto deferente, cauda do epidídimo e glândula vesicular) foram coletadas de 42 touros abatidos. Teste de soroaglutinação microscópica (SAM), reação em cadeia da polimerase (PCR) e isolamento microbiológico foram incluídos como métodos de diagnóstico. Anticorpos anti-*Leptospira* spp. foram encontrados em 17 (40,48%) animais, enquanto 26 animais (61,90%) apresentaram pelo menos um órgão ou urina com DNA leptospírico, e 10 animais (23,81%) foram positivos na cultura bacteriológica. As amostras sequenciadas a partir do gene *LipL*32 apresentaram 99% de similaridade com *Leptospira borgpetersenii*. Recomenda-se a análise molecular de amostras de ducto deferente e cauda do epidídimo para o diagnóstico de leptospirose genital em touros e, na impossibilidade da coleta desses tecidos, o sêmen pode ser utilizado. Em conclusão, este estudo fornece informações importantes sobre touros do bioma Caatinga, Brasil, como portadores de leptospirose genital. Os resultados indicam que, mesmo em condições ambientais adversas, as leptospiras podem sobreviver e se propagar, principalmente devido à característica de os portadores genitais disseminarem sexualmente espécies adaptadas de *Leptospira* sem influência de variáveis externas. Assim, as estratégias de prevenção e controle da leptospirose bovina precisam incluir ações voltadas para o portador genital.

TERMOS DE INDEXAÇÃO: *Leptospira* spp., semiárido, PCR, cultura bacteriológica, portador genital, touros, Caatinga.

INTRODUCTION

Leptospirosis is a disease that causes economic and social impact, as it affects domestic and wild animals and humans (Adler 2015). The etiological agent, a pathogenic species of *Leptospira* bacteria, is capable of rearranging its genome in the process of adaptation to new hosts, resulting in a complex epidemiological chain. Such dynamism makes the prevention and control of this zoonosis remain challenging (Picardeau 2017). Exposure occurs from direct contact with an infected animal or indirectly through water and soil contaminated by urine (Gómez-Martín et al. 2023). In animals, transmission also occurs from contact with vaginal fluid and placental remains, during mating and vertically (Lilenbaum et al. 2008).

In livestock, leptospirosis causes economic losses due to abortions, weak offspring, retarded growth, low milk production, or agalactia and death (Mughini-Gras et al. 2014). In cattle, economic losses are more related to early embryonic loss and repetition of estrus. Although in the bovine species, it is a reproductive disease and bovine genital leptospirosis (BGL) has been proposed, most studies use urinary tract samples for direct diagnosis and the genital tract is underused, which is nonsense as genital biological materials should be of choice for diagnosing the chronic form of the disease (Loureiro & Lilenbaum 2020, Di Azevedo & Lilenbaum 2021). In addition, there is no report regarding the role of bulls in the epidemiology of BGL, mainly in semiarid conditions such as the Caatinga biome.

Cattle are reservoirs of serogroup Sejroe and can host leptospires for a long time (Loureiro & Lilenbaum 2020). Infections in humans with strains of serogroup Sejroe have been reported (Allan et al. 2020, Grillová et al. 2020), thus, bovine can be a source of infection for humans, which constitutes a concern for One Health (Barnabé et al. 2023). Investing in the prevention and control of diseases such as leptospirosis results in increased livestock production, in addition to reducing field worker exposure to the pathogen. According to Pimenta et al. (2019), strategies must consider factors inherent to the agent, the host, and the environment, Martins & Lilenbaum (2017) stated that each herd has particularities and needs specific measures that address the identification of carriers, treatment, and vaccination.

The semiarid region of Brazil is characterized by low rainfall and high temperatures and is located in the Caatinga, an exclusively Brazilian biome that, with its peculiar vegetation and abundant fauna, offers unique epidemiological conditions that can influence the occurrence of diseases. Considering that in bovine leptospirosis the genital carrier is constantly neglected, this study aimed to investigate bulls maintained in Caatinga biome conditions as genital carriers of *Leptospira* spp., using the main methods for the diagnosis of leptospirosis – microscopic agglutination test (MAT), polymerase chain reaction (PCR) and microbiological isolation.

MATERIALS AND METHODS

Ethical statement. All experimental protocols were approved by the Animal Ethics Committee (CEUA) of the "Universidade Federal de Campina Grande" (UFCG), protocol ID# 069-2018. All procedures were undertaken according to the relevant guidelines and regulations.

Study area. Biological samples were collected from the public slaughterhouse of Patos County (latitude: 7°00'19'' South; longitude: 37°16'48'' West) in the state of Paraíba, Northeastern Brazil. The animals came from areas located in the Caatinga biome and were slaughtered within a maximum period of two days. The Caatinga is an exclusively Brazilian biome, with a semiarid climate, high solar radiation, with long periods of water scarcity which has stunted vegetation. The climate is hot and dry, with rains concentrated in summer/autumn between March and April. However, rainfall can occur between January and May. Drought can last for more than a year, resulting in a negative water balance (Alvares et al. 2014). The period in which the present study was carried out corresponded to the dry season, with average rainfall and temperature of 6.70mm and 30.10°C, respectively (INMET 2021).

Sampling. The minimum sample size was determined using the following formula for proportion analysis (Arango 2009):

$$n = \frac{p_0 \times q_0 \left(z_{1-\beta} + z_{\alpha/2} \times \sqrt{\frac{p_1 \times q_1}{p_0 \times q_0}} \right)^2}{(p_1 - p_0)}$$

Where: n = minimum sample size; $Z_{\alpha/2}$ = 1.96 (Z value for 95% confidence level); $Z_{1-\beta}$ = 1.64 (Z value for power of 95%); P_0 = 33%

(reference proportion for PCR positivity) (Pimenta et al. 2019); P_1 = 61.40% (estimate for the experimental proportion of positivity in PCR) (Loureiro et al. 2017); $q0 = 1-p_0$; $q1 = 1-p_1$.

According to these parameters, a minimum of 37 animals was needed to assess true prevalence within a confidence interval of 95%; however, 42 cattle were used. They were all male, aged greater than or equal to 24 months, cross-bred, and had no history of vaccination against leptospirosis. According to the data taken from animal movement forms held by the State Veterinary Service of Paraíba, these male cattle came from rural farms located in the semiarid region, from municipalities belonging to two federative units: Paraíba (Cacimba de Areia, Cacimbas, Condado, Passagem, Patos, Pombal, Quixaba, São José de Espinharas e São Mamede) and Pernambuco (São Bento do Una).

Biological sample collection. Blood samples were collected from jugular veins using 8ml labeled sterile tubes containing a coagulation activator just prior to slaughter of the animals. After collection, the tubes were sent to the laboratory, where they were centrifuged at 1,512g for 10 min, and the serum samples were stored in microtubes at -20ºC. Bladder, kidney, vas deferens, cauda epididymis, and vesicular gland samples were collected for direct diagnosis of *Leptospira* spp. infection. The tissues were immediately fragmented by using autoclaved surgical materials and sterile slides for each tissue. After that, the fragments were immediately transferred to a specific room in the slaughterhouse, where there was a Bunsen burner, and were deposited onto autoclaved Petri dishes while avoiding contact between the fragments. The material was fragmented into smaller portions of ≈2gm (in duplicate); one part was immediately seeded into the culture medium, while another part was placed into DNA and RNA-free microtubes and stored at -20ºC for molecular detection. The urine was obtained by cystocentesis during evisceration (in duplicate), using 5mL sterile syringes, three drops being immediately seeded in culture medium and another fraction of urine stored in DNA- and RNA-free microtubes with 0.5mL of phosphate-buffered saline (Nogueira et al. 2020).

Microscopic agglutination test (MAT). The MAT was used to detect anti-*Leptospira* spp. antibodies using a collection of 24 serovars belonging to 17 different pathogenic serogroups of five species provided by the Laboratory of Veterinary Bacteriology of the "Universidade Federal Fluminense" (UFF), Niterói, Rio de Janeiro, Brazil, originating from the Pasteur Institute, France. The *Leptospira* species and serovars were *L. interrogans*: Copenhageni, Canicola, Autumnalis, Wolffi, Hardjoprajitno, Icterohaemorrhagiae, Pomona, Kennewicki, Hebdomadis, Pyrogenes, Bratislava and Australis; *L. santarosai*: Guaricura, Shermani, and Canalzoni; *L. borgpetersenii*: Javanica, Tarassovi, Ballum, Mini and Castellonis; *L. kirschneri*: Grippotyphosa and Cynopteri; *L. noguchi*: Panamá and Lousiana (WOAH 2023). Following the recommendation for diagnosing leptospirosis in cattle maintained in the Caatinga biome, a cutoff point of 50 was adopted (Barnabé et al. 2023). Sera were sorted, the positive ones being double diluted, obtaining as a final result the respective highest titer achieved (WOAH 2023).

Microbiological culture. Immediately after collection, approximately 2gm of each tissue and three drops of urine were inoculated into tubes containing 5mL of liquid EMJH medium (Difco, BD Franklin Lakes/NJ, USA) with amphotericin B (0.05mg/mL), 5-fluorouracil (0.1mg/mL), fosfomycin (0.4mg/mL), trimethoprim (0.2mg/mL) and sulfamethoxazole (0.4mg/mL) (Chakraborty et al. 2011). After 24 hours, 1ml of the primary culture was inoculated into a semi-solid EMJH medium without antibiotics and then kept in a biological oxygen demand incubator (BOD) at 30ºC. Regardless of the presence of the Dinger ring zone, the tubes were examined weekly for 12 weeks using dark-field microscopy.

Polymerase chain reaction (PCR) and sequencing. The Dneasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used to extract DNA from tissues and urine, as well as from cultures in EMJH that showed leptospires growth as per dark-field microscopy examination, according to the manufacturer's recommendations. The gene *LipL*32, specific to pathogenic leptospires and, therefore, of public health importance, was amplified with *LipL*32-45F (50'-AAG CAT TAC CGC TTG TGG TG-3') and *LipL*32-286R (50'-GAA CTC CCA TTT CAG CGA TT-3') primers (Stoddard et al. 2009) following the procedures for PCR previously described (Hamond et al. 2014). Primers were used in a concentration of 0.6µM, 1.0U *Taq* polymerase, 2.4µM MgCl2, 0.3mM dNTP in a final volume of 25µL. One cycle of initial denaturation at 94ºC for two minutes, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing the primers to 53ºC for 30 s and one-minute extension with 72ºC and a final extension cycle at 72ºC for five minutes were used. PCR products were developed by 2% ultrapure agarose gel electrophoresis stained with Evans Blue (Thermo Fisher Scientific, Waltham/MA, USA) and 100bp ladder, and DNA bands (242bp) were visualized under ultraviolet light. Strain *Leptospira interrogans* serovar Copenhageni, Fiocruz L1-130 (ATCC BAA-1198) was used as the positive control, and ultrapure water was used as a negative control.

*LipL*32-45F and *LipL*32-286R primers (Stoddard et al. 2009) were used in the sequencing reactions with the Kit Big Dye Terminator v3.1 (Applied Biosystems, Foster City/CA, USA). 3130xl Genetic Analyzer and POP-7 polymer (Platt et al. 2007) were used for capillary electrophoresis, sequence alignment was conducted by using BioEdit (Gouy et al. 2010), and the dataset strings were obtained from GenBank (National Biotechnology Information Center, Bethesda/MD, USA)[6] using the BLAST tool[7]. SeaView4 software (Hall 1999) was applied during the phylogenetic analysis, and the neighbor's association model was used to build a phylogenetic tree with a bootstrap value of 1,000 repetitions[8], as viewed through the FigTree v1.4.3[9]. The phylogenetic reconstruction included *Leptospira* sequences for comparison.

Statistical analysis. The proportions of positive animals and samples were compared by using the chi-squared test with Yates' continuity correction or Fisher's exact test using the BioEstat 5.3 software (Ayres et al. 2007) with a 5% significance level ($P \leq 0.05$).

RESULTS

Leptospira spp. antibody detection

Seventeen out of the 42 animals (40.48%; 95% CI = 27.04%-55.51%) presented anti-*Leptospira* spp. antibodies and the reactive serogroups were Sejroe, Tarassovi, Canicola, and Grippotyphosa. Two animals were seroreactive for Sejroe and Tarassovi at titer (50), one animal reacted for Sejroe at titer 100, two animals reacted for Sejroe at titer 200, seven animals reacted for Tarassovi, Grippotyphosa, Canicola and Sejroe at titer 400, two animals reacted for Sejroe and Tarassovi at titer 800, two animals reacted for Sejroe and Tarassovi at titer 1600, and one animal was seroreactive for Sejroe at titer 3200 (Table 1).

[6] Available at <http://www.ncbi.nlm.nih.gov> Accessed on Nov. 19, 2022.

[7] Available at <http://www.ncbi.nlm.nih.gov/BLAST/> Accessed on Nov. 19, 2022.

[8] Available at <http://tree.bio.ed.ac.uk/software/figtree/> Accessed on Nov. 26, 2022.

[9] Available at <http://tree.bio.ed.ac.uk/> Accessed on Nov. 26, 2022.

Leptospira spp. DNA detection

Leptospiral DNA was found in at least one sample in 26 animals (61.90%; 95% CI = 46.81%-75.00%). Among the 252 samples, PCR detected leptospire DNA in 86 (34.13%). The most frequent PCR-positive samples were the vas deferens (19 samples; 45.24%), kidney (17 samples; 40.48%), bladder (15 samples; 35.71%), and cauda epididymitis (15 samples; 35.71%). There were statistically significant differences ($P<0.05$) between urine and vas deferens and urine and kidney. Leptospire-specific DNA was identified in 16 cultures (Table 2).

Table 1. Results of serological, molecular and microbiological tests for *Leptospira* spp. infection in bulls from the Caatinga biome, Brazil

Animal	MAT	PCR						Microbiological culture					
	17*/42 (40.48%)	26*/42 (61.90%)						24*/42 (57.14%)					
	Serogroup (titer)	UR	BL	KID	VD	CE	VG	UR	BL	KID	VD	CE	VG
1	-	+	+	+	+	+	+	+	+ +	-	+	+	+ +
2	Sejroe (3200)	-	+	+	+	+	+▲	-	+ +	+ +	+	+	+ +
3	-	-	+	+	+	+	+	-	+ +	-	++	-	+
4	Tarassovi (400)	-	+	-	+	+	+	-	+ +	-	-	++	-
5	-	+	-	+	-	-	-	+	-	-	-	-	-
6	Sejroe (200)	-	-	-	-	-	+	-	-	-	-	-	+
7	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Sejroe (800)	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	+	-	-	-	-	-	-	-	-
10	Sejroe (100)	-	+	+	+	+	+	-	+	+	+	+	+
11	-	+	+	+	+	+	+	+ +	+	+	+	+	+
12	Grippotyphosa (400)	+	+	+	+	-	-	+	-	+ +	-	-	-
13	-	+	+	+	+	-	-	-	-	+	+	-	-
14	-	+	+	+	+	-	-	-	-	+	-	-	-
15	-	-	-	+	-	-	-	-	-	+	-	-	-
16	-	-	+	-	-	-	-	-	+	-	-	-	-
17	Sejroe (200)	-	+	-	+	+	-	-	+ +	-	+	+	-
18	-	-	-	+	-	+	-	-	-	+	-	+	-
19	-	-	-	-	+	-	-	-	-	-	++	-	-
20	-	-	+▲	+	+	+	+	-	+ + ♦	-	+	-	+
21	Canicola (400)	-	-	-	-	-	-	-	-	-	-	-	-
22	Sejroe (1600)	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-
24	Tarassovi (800)	-	-	-	-	-	-	-	-	-	-	-	-
25	Sejroe (400)	-	-	-	-	+	-	-	-	-	-	+	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	+	+	+	+	+	+	+	+	+	+	+	+
29	-	-	-	-	+	-	-	-	-	-	-	-	-
30	Sejroe (400)	-	+	+	+	+	+	-	-	+	+	+	+
31	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	+	+	-	-	-	-	+	+	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-	-	-	-
35	Sejroe (50)	-	-	-	-	-	-	-	-	-	-	-	-
36	Tarassovi (50)	-	-	-	-	-	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-	-	-	-	-	-	-
38	Sejroe (400)	-	-	-	-	-	-	-	-	-	-	-	-
39	Tarassovi (1600)	+	-	+	-	-	-	+	-	+	-	-	-
40	-	-	-	-	-	-	-	-	-	-	-	-	-
41	Sejroe (400)	-	-	+	+	+	+	-	-	+	+	+	+
42	-	-	+	+	+	+	+	-	+ +	+	+	+	+ +
Total positives	17	8	15	17	19	15	12	6	11	13	14	13	11

* Number of positive animals; UR = urine, BL = bladder, KID = kidney, VD = vas deferens, CE = cauda epididymidis, VG = vesicular gland; (-) negative sample, (+) positive sample, (+ +) microbiological culture positive at PCR, ▲ sample sequenced from tissue, ♦ sample sequenced from culture.

Microbiological culture

The pathogen was visualized in at least one sample in 24 animals (57.14%; 95% CI = 42.21%-70.88%), and 68 cultures (26.98%) out of 252 were found to be positive, highlighting the vas deferens (14 samples; 33.33%), cauda epididymitis (13 samples; 30.95%) and kidney (13 samples; 30.95%). Urine differed statistically ($P<0.05$) from the vas deferens. *Leptospira* spp. DNA was identified in at least one microbiological culture in 10 animals (23.81%; 95% CI = 13.48%-38.53%), and the biological materials with the highest frequencies were bladder (7 samples; 16.67%) and vesicular gland (3 samples; 7.14%) (Table 2).

Leptospiral DNA sequencing

Due to budget issues, DNA sequencing from the PCR products was possible in two samples taken from tissues (vesicular gland and bladder) from different animals and one from culture (bladder). These samples showed a 99% similarity with *Leptospira borgpetersenii* (Fig.1) strains R14-L chromosome 1, R6L chromosome 1, R14 chromosome 1, R6 chromosome 1, Mo4 chromosome 1, R23 chromosome 1, R28 chromosome 1 and R29 chromosome 1.

DISCUSSION

The high frequency of seroreactivity found (40.48%) indicates that, even under adverse climatic conditions, *Leptospira* spp. can be present in herds in the Caatinga biome. There was variation in reactive serogroups (Sejroe, Tarassovi, Canicola e Grippotyphosa), which suggests different sources of infection, although there is a possibility that cattle without signs and symptoms of infection may carry and disseminate other strains within the species in an adaptive process (Pinto et al. 2017). As in other regions of Brazil (Hashimoto et al. 2017, Miashiro et al. 2018, Pinna et al. 2018, Guedes et al. 2019a, 2019b, Pimenta et al. 2020) and the world (Loureiro et al. 2016, Pinto et al. 2016), Sejroe was the most prevalent serogroup in Caatinga biome cattle.

The contrast between unfavorable climatic conditions (average rainfall and temperature of 6.70mm and 30.10ºC, respectively, in the dry season) and a significant proportion of seroreactive animals, especially regarding the Sejroe serogroup, provides evidence for intraspecies transmission, less dependent on environmental factors because cattle are maintenance hosts (Ellis 2015). Tarassovi is one of the main serogroups found in cattle worldwide (Soo et al. 2020), having been reported in pigs (Fernandes et al. 2020a) and "teiús" (*Tupinambis merianae*) from the Brazilian semiarid region (Fernandes et al. 2020b). The reservoir of the Canicola serogroup is the domestic dog (*Canis lupus familiaris*) (Goarant 2016), but it is also common in small wild mammals (Guedes et al. 2019b). In Brazil, a study from the 1990s suggests the rat (*Rattus norvegicus*) and the marsupial (*Didelphis marsupialis*) as natural hosts of serogroup Grippotyphosa (Caldas et al. 1992).

Leptospiral DNA was detected in 61.90% of the animals and when comparing the positivity rates between different biological materials, there were statistically significant differences ($P<0.05$), demonstrating that leptospires accumulate preferentially in the vas deferens when compared to urine. This may be explained by leptospire's small size, helical morphology, and translational motility that facilitate access to organs, allowing the immune system to escape due to the physical barrier that hinders the contact between antibody molecules and the antigen, as well as the highlight of the vas deferens may be related to the lower amount of inhibitors of *Taq* DNA polymerase enzyme, such as chelation of free magnesium ions, hemoglobin, bile salts and acid polysaccharides of glycoproteins (Genovez et al. 2006); or simply reflect the release of bacteria in secretions of the epididymis and testis (seminiferous tubules), while leptospiruria occurs intermittently. The broad presence of microorganisms in the reproductive tract reinforces that this is a site of extra renal colonization in cattle, which can act as adapted hosts (Loureiro et al. 2017, Pires et al. 2018). PCR results indicate that semen can be valuable in identifying the genital carrier.

Leptospira spp. was PCR-confirmed in microbiological cultures from 10 animals (23.81%) and 16 samples (6.35%). By comparing the positivity rates between the different materials, there was no statistical difference probably due to the few animals and samples analyzed.

The sequenced DNA from three samples demonstrated 99% similarity with *Leptospira borgpetersenii*, which belongs to the pathogenic clade and, according to virulence, to subgroup 2, along with the species *L. santarosai*, *L. mayottensis*, *L. weilii* and *L. alexanderi*. Adapted to cattle, *L. borgpetersenii* has the smallest genome compared to other pathogenic and saprobic strains, which determines its low resistance to the environment (Bulach et al. 2006). This species also causes early embryonic loss and estrus repetition, resulting from uterine inflammation and damage caused by embryo invasion

Table 2. *Leptospira* spp. molecular and microbiological diagnoses according to different types of biological material from bulls from the Caatinga biome, Brazil

Sample	Number of samples	PCR	Microbiological culture	PCR from culture
		26*/42 (61.90%)	24*/42 (57.14%)	10*/42 (23.81%)
		+ (%)	+ (%)	+ (%)
Urine	42	8 (19.1) [a]	6 (14.29)	1 (2.38) [a]
Bladder	42	15 (35.71) [ab]	11 (26.19)	7 (16.67) [a]
Kidney	42	17 (40.48) [b]	13 (30.95)	2 (4.76) [a]
Vas deferens	42	19 (45.24) [b]	14 (33.33)	2 (4.76) [a]
Cauda epididymidis	42	15 (35.71) [ab]	13 (30.95)	1 (2.38) [a]
Vesicular gland	42	12 (28.57) [ab]	11 (26.19)	3 (7.14) [a]
TOTAL	252	86 (34.13)	68 (26.98)	16 (6.35)

* Number of positive animals; (+) positive samples; [a,b] Different lowercase letters in the same column indicate significantly different proportions ($P\leq0.05$).

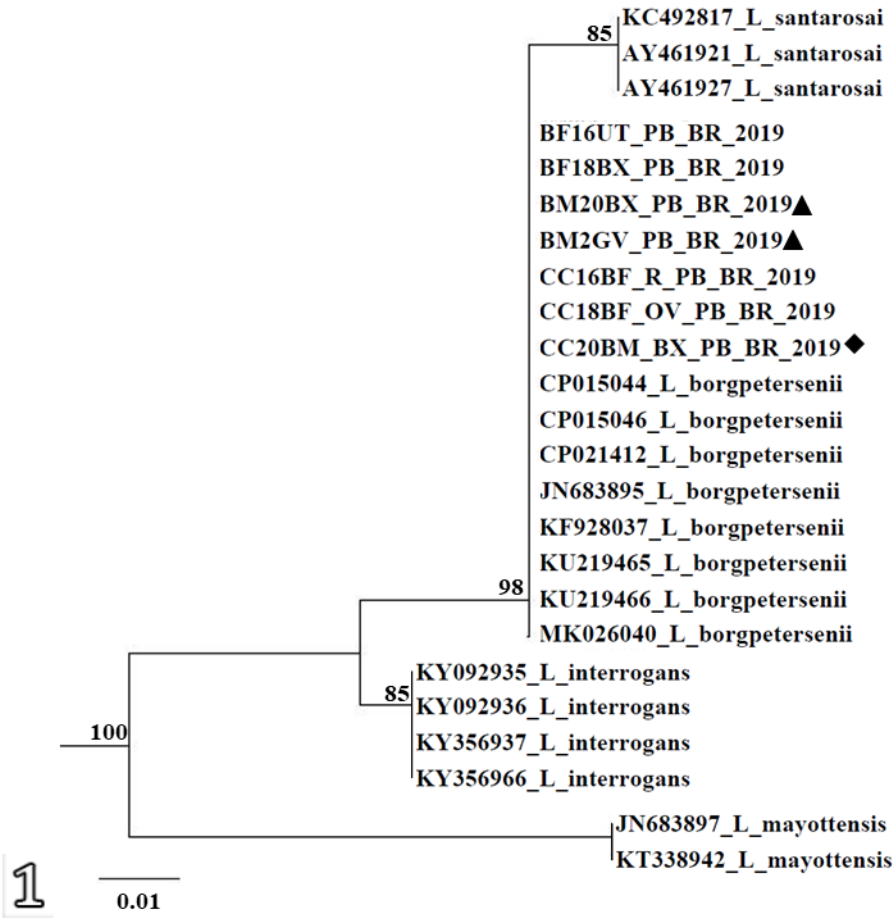

Fig.1. Phylogenetic tree based on the alignment of nucleotide sequences of the *LipL32* gene *Leptospira* sp., constructed using the neighbor-joining model with 1000 replicas. Sample sequenced from culture (♦), sample sequenced from tissue (▲).

(Loureiro & Lilenbaum 2020). In addition, this species also infect humans (Grillová et al. 2021), which implies the need for integrated actions (One Health) to monitor and control leptospirosis.

Out of the 26 positive animals on PCR, 16 showed positivity in both the reproductive and urinary tracts, six were positive only in the reproductive tract and four only in the urinary tract. Bulls with urogenital tract involvement potentiate the diffusion of the agent, especially in the rainy season of a semiarid region, due to the possibility of simultaneous transmission via the urinary and venereal routes.

Bovine genital leptospirosis has been proposed as a syndrome dissociated from systemic and renal disease. Its diagnosis should be based on the detection of leptospires in samples from the genital tract, and treatment requires further research (Loureiro & Lilenbaum 2020). Most studies have investigated cows as carriers, consequently, there is little information about carrier bulls. There are gaps in the symptomatology and the level of reproductive impairment, such as decreased libido, damage to sperm and sperm viability, or whether only the male acts as a venereal disseminator of the microorganism. It should be noted that transmission also occurs by artificial insemination, a warning about the importance of considering this syndrome when sorting animals for assisted reproduction biotechniques (Pereira et al. 2022).

Genital leptospirosis is also researched in other animal species and the results can serve as guidance for studies in the bovine species. Di Azevedo & Lilenbaum (2022) investigated this syndrome in equines and highlighted that control through vaccination and antibiotic therapy is uncertain. Martins et al. (2022) proved in sheep that, despite vaccination, leptospires colonize the genital tract. Guadelupe et al. (2022) found that the therapeutic protocol recommended for leptospirosis in ruminants, a single dose of streptomycin (25mg/kg, IM), is ineffective in eliminating the genital tract infection. However, when treatment was extended to three consecutive days, at the same dose, the drug was 100% effective in eliminating genital carrier status in sheep.

In the Caatinga biome, where climatic conditions are adverse to bacterial survival in the environment, extra renal colonization by *L. borgpetersenii* is a strong indication of venereal transmission as an alternative route, unlike urinary transmission, which depends on the environment. The explanation for a high frequency of positive bulls during the dry period may lie in genital leptospirosis and venereal transmission. Therefore, in the dry season, the urinary tract may be less relevant for the spread of the pathogen, except in microclimates, which would be unlikely in the present study, considering that the animals lived on different farms".

CONCLUSION

This study provides important information relating to bulls from the Caatinga biome, Brazil, as carriers of genital leptospirosis. The results indicate that, even under adverse environmental conditions, *Leptospira* spp. may survive and propagate, mainly due to the characteristic of genital carriers for the sexually spreading of adapted *Leptospira* species without influence by external variables. Thus, prevention and control strategies for bovine leptospirosis need to include actions aimed at the genital carrier.

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