PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 44:e07482, 2024
DOI: 10.1590/1678-5150-PVB-7482

Original Article
Livestock Diseases

Evidence and implications of pigs as genital carriers of *Leptospira* spp. in the Caatinga biome[1]

Hosaneide G. Araújo[2], Vitória V.F. Aquino[2], Luiz F.A. Pedrosa[2], Clebert J. Alves[2], Maria L.C.R. Silva[2], Vinícius L.R. Vilela[3], João P. Araújo Júnior[4], Camila D. Malossi[4], Carolina S.A.B. Santos[2] and Sérgio S. Azevedo[2]*

ABSTRACT.- Araújo H.G., Aquino V.V.F., Pedrosa L.F.A., Alves C.J., Silva M.L.C.R., Vilela V.L.R., Araújo Júnior J.P., Malossi C.D., Santos C.S.A.B. & Azevedo S.S. 2024. **Evidence and implications of pigs as genital carriers of *Leptospira* spp. in the Caatinga biome**. *Pesquisa Veterinária Brasileira 44:e07482, 2024*. Unidade Acadêmica de Medicina Veterinária, Universidade Federal de Campina Grande, Av. Universitária 110, Patos, PB 58708-110, Brazil. E-mail: sergio.santos@professor.ufcg.edu.br

The Caatinga biome is unique to Brazil, with unfavorable environmental characteristics for the survival of *Leptospira* spp. However, recent studies have shown high positivity at PCR (polymerase chain reaction) in small ruminants. There are no *Leptospira* spp. studies based on sample calculation in pigs in the Caatinga. The aim of this study was to assess the importance of pigs in the spread of leptospirosis in the Caatinga biome. Overall, 200 biological samples (urine, blood, vaginal fluid, and tissues of reproductive and urinary tracts) were collected from 40 slaughtered sows, and MAT (microscopic agglutination test) and PCR tests were carried out to detect anti-*Leptospira* spp. antibodies and the agent's DNA, respectively. The serological analysis showed a positivity rate of 5% (2/40), and the PCR identified *Leptospira* spp. DNA in 62.5% (25/40) of the animals. Only 2.5% (1/40) of the animals were positive for both techniques. The detected serogroups were Australis (50%) and Bataviae (50%), with antibody titers of 25 and 50. *Leptospira* spp. DNA was detected in 40% (16/40) of the reproductive tract samples, 32.5% (13/40) of the urinary tract, 32.5% (13/40) of the vaginal fluid and 30% (12/40) of the urine. There was no agreement (Kappa <0) between PCR samples from the genital tract vs. urinary tract or serological results. Genetic sequencing of one urine and one urinary tract tissue sample revealed 99% identity with *L. borgpetersenii*. The results indicate that leptospirosis is a concern in pigs in the context of Caatinga, with a high prevalence of infection detected by different diagnostic methods. The molecular analysis revealed a considerable proportion of infected animals. The findings emphasize the importance of a multifaceted approach in the diagnosis of leptospirosis in pigs, with a focus on the use of genital tract samples for the diagnosis of leptospirosis in this animal species, providing valuable insights for the control and prevention of this disease in both animals and the zoonotic context. Finally, the detection of leptospires in the genital tract indicates a possibility of male-female transmission in the venereal context.

INDEXING TERMS: Leptospires, pigs, epidemiology, One Health, semiarid.

[1] Received on April 29, 2024.
Accepted for publication on May 14, 2024.
[2] Unidade Acadêmica de Medicina Veterinária (UAMV), Universidade Federal de Campina Grande (UFCG), Av. Universitária 110, Patos, PB 58708-110, Brazil. *Corresponding author: sergio.santos@professor.ufcg.edu.br

[3] Instituto Federal de Educação Ciência e Tecnologia da Paraíba (IFPB), Rua Presidente Tancredo Neves s/n, Sousa, PB 58805-345, Brazil.

[4] Instituto de Biotecnologia (IBTEC), Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), Alameda das Tecomarias s/n, Botucatu, SP 18607-440, Brazil.

RESUMO.- [Evidência e implicações de suínos como carreadores genitais de *Leptospira* spp. no bioma Caatinga.] O bioma Caatinga é único no Brasil, com características ambientais desfavoráveis à sobrevivência de *Leptospira* spp. Porém, estudos recentes demonstraram alta positividade na PCR (reação em cadeia da polimerase) em pequenos ruminantes. Não existem estudos para a infecção por *Leptospira* spp. baseados em cálculo amostral em suínos na Caatinga. O objetivo deste estudo foi avaliar a importância dos suínos

na disseminação da leptospirose no bioma Caatinga. Foram coletadas 200 amostras biológicas (urina, sangue, fluido vaginal e tecidos do trato reprodutivo e urinário) de 40 porcas abatidas e realizados testes SAM (teste de soroaglutinação microscópica) e PCR para detecção de anticorpos anti-*Leptospira* spp. e DNA do agente, respectivamente. A análise sorológica mostrou taxa de positividade de 5% (2/40) e a PCR identificou o DNA de *Leptospira* spp. em 62,5% (25/40) dos animais. Apenas 2,5% (1/40) dos animais foram positivos para ambas as técnicas. Os sorogrupos detectados foram Australis (50%) e Bataviae (50%), com títulos de anticorpos de 25 e 50. O DNA de *Leptospira* spp. foi detectado em 40% (16/40) das amostras do trato reprodutivo, 32,5% (13/40) do trato urinário, 32,5% (13/40) do fluido vaginal e 30% (12/40) de urina. Não houve concordância (Kappa <0) entre amostras de PCR do trato genital vs. trato urinário ou resultados sorológicos. O sequenciamento genético de uma amostra de urina e de uma amostra de tecido do trato urinário revelou 99% de identidade com *L. borgpetersenii*. Os resultados obtidos indicam que a leptospirose representa uma preocupação em suínos no contexto da Caatinga, com alta prevalência de infecção detectada por diferentes métodos diagnósticos, bem como análises moleculares revelaram proporção considerável de animais infectados. Os resultados enfatizam a importância de uma abordagem multifacetada no diagnóstico da leptospirose em suínos, com foco no uso de amostras do trato genital para o diagnóstico da leptospirose nesta espécie animal, fornecendo informações valiosas para o controle e prevenção desta doença em animais e no contexto zoonótico. Por fim, a detecção de leptospiras no trato genital indica possibilidade de transmissão macho-fêmea no contexto venéreo.

TERMOS DE INDEXAÇÃO: Leptospiras, suínos, epidemiologia, Saúde Única, semiárido.

INTRODUCTION

Leptospirosis is a worldwide zoonosis caused by pathogenic species of *Leptospira* spp., and a large number of mammals are susceptible to the disease, including farm animals and humans. The infection is of economic importance in pigs worldwide due to reproductive losses such as abortions, infertility, stillbirths and the birth of weak piglets (Zimmerman et al. 2019, Steinparzer et al. 2021). It is also considered an occupational disease; humans with direct contact with sick or carrier pigs can get infected. It is most frequently associated with veterinarians, livestock farmers and slaughterhouse employees (Gonçalves et al. 2021).

The occurrence of animal and human leptospirosis is facilitated by management practices, human behavior and environmental factors. Due to its ability to infect multiple hosts and reservoirs, *Leptospira* spp. plays an important role in the human-animal-environment interface (Petrakovsky et al. 2014, WHO et al. 2019), so a multisectoral One Health approach is needed to understand the relationship between infection in humans and animals, as well as the role of the environment in transmission (Ospina-Pinto & Hernández-Rodríguez 2021).

Rodents, small marsupials, cattle, pigs and dogs are deemed important sources of infection, and people living in rural areas are at greater risk, especially in tropical countries, where they are in close contact with environments inhabited by sources of infection (Adler & de la Peña Moctezuma 2010, Araújo et al. 2023). Previous reports indicate that pigs act as maintenance hosts for the Bratislava, Pomona and Tarassovi serovars, while among the incidental serovars, the most important in pigs are those belonging to Icterohaemorrhagiae, Canicola and Grippotyphosa serogroups (Ellis 2015, Bertasio et al. 2020).

Currently, based on phylogenetic analyses, *Leptospira* spp. is divided into three lineages that constitute the level of pathogenicity: saprophytic (26 species), intermediate (21 species) and pathogenic (17 species). The intermediate species share an almost common ancestor with the pathogenic species, although they exhibit moderate pathogenicity in humans and animals (Vincent et al. 2019).

The Caatinga biome occurs only in Brazil and has characteristics of dry forests, high temperatures and low humidity, as well as broad biodiversity. It covers an area of 826,411km^2 (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 (Embrapa 2022). 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).

The diagnosis of leptospirosis is based on clinical examination and serological and molecular tests. Among all the serological tests used, the microscopic serum agglutination test (MAT) is considered the gold standard (Rajapakse et al. 2020). Despite being the reference method for diagnosing leptospirosis, MAT has limitations, including low sensitivity in the acute phase of the disease and inability to differentiate IgM from IgG antibodies (Rajapakse et al. 2015). In addition, MAT is a laborious and expensive technique due to the need to maintain live bacteria as antigens (Padilha et al. 2022).

There is no *Leptospira* spp. survey in the Caatinga based on sample calculation and analysis of possible alternative routes of transmission of leptospirosis in swine, so the aim of this study was to evaluate the importance of pigs in the spread of leptospirosis in the Caatinga biome and to identify possible alternative routes of transmission of the pathogen, using serological and molecular techniques.

MATERIALS AND METHODS

Animal Ethics. All experimental protocols were approved by the Animal Ethics Committee (CEUA) of the "Universidade Federal de Campina Grande", protocol ID# 30-2019. All procedures were undertaken in accordance with the relevant guidelines and regulations.

Sampling and biological sample collection. This research was carried out at the Patos Municipal Public Slaughterhouse (Latitude: 07°01'28" S; Longitude: 37°16'48" W), located in the Caatinga biome in the semiarid of Paraíba state, Northeast region of Brazil. The biological samples were collected in June and July 2021, corresponding to the dry season's beginning. The production system in the region is characterized by family subsistence farming, where there is no effective sanitary control. According to the Animal Transit Guides provided by the Official Veterinary Service of the State of Paraíba, all

animals came from different rural properties located in the Caatinga biome and these properties did not vaccinate against leptospirosis.

The minimum sample size was determined using the following formula for analyzing proportions (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 confidence level of 95%)

$Z_{1-\beta}$ = 1.64 (Z value for 95% statistical power)

P_0 = 22% (reference proportion for PCR-positivity) (Fernandes et al. 2020)

P_1 = 61.40% (estimative for the experimental proportion of PCR) (Loureiro et al. 2017)

$q_0 = 1-p0$

$q_1 = 1-p1$

According to these parameters, 18 animals would have been needed; however, 40 sows were used. Overall, 200 samples were collected from 40 animals, including 40 blood samples, 40 urine samples, 40 vaginal fluid samples, 40 reproductive tissue samples (uterus, uterine tube and ovary) and 40 urinary tissue samples (kidney and bladder). The blood samples were collected on the slaughter line during the bleeding of the animals, using sterile tubes with a coagulation activator and a capacity of 8mL. The tubes were then transported to the laboratory, where they were centrifuged at 1,512g for 10 minutes, and the serum samples were stored in microtubes at -20°C.

For molecular diagnosis of *Leptospira* spp. fragments of the urinary tract (kidney and bladder) and reproductive tract (ovary, uterus and uterine tube) were collected from pools of each animal using surgical scissors, sterile anatomical forceps and a scalpel with a disposable carbon steel blade. The fragments were then immediately transferred to a specific room in the slaughterhouse, where there was a Bunsen burner, and placed into autoclaved Petri dishes without contact between the fragments. These pools were immediately fragmented and placed in quantities of approximately two grams (in duplicates) in DNA- and RNA-free microtubes and stored at -20°C for later molecular detection. In addition to the pools of tissues, vaginal fluid was also collected with sterile swabs directly from the cervix and urine by cystocentesis during evisceration, using sterile 5mL syringes. Both samples were also stored in duplicate in DNA- and RNA-free microtubes and the swabs were added to a lysis solution to preserve and stabilize the proteins.

Microscopic agglutination test (MAT). The detection of anti-*Leptospira* spp. antibodies were carried out using the microscopic serum agglutination test (MAT), using a collection of 17 serovars from five different species: *Leptospira interrogans* serovars Canicola, Wolffi, Hardjoprajitno, Icterohaemorrhagiae, Pomona, Hebdomadis, Bratislava, Bataviae, Djasiman and Australis; *L. borgpetersenii* serovars Javanica, Tarassovi, Mini and Castellonis; *L. kirschneri* serovar Grippotyphosa; *L. noguchii* serovar Lousiana; *L. biflexa* serovar Patoc. MAT was carried out according to the World Organisation for Animal Health standards (WOAH 2021). Each serum sample was initially diluted 1:25 (cut-off point 25) in buffered saline solution pH 7.2, and samples that showed 50% or more agglutination were considered positive. Positive samples were two-fold serially diluted, and the highest titer obtained was considered to identify the probable infecting serogroup.

***Leptospira* spp. molecular detection and sequencing.** DNA was extracted from urine, vaginal fluid and tissue pools from the urinary tract (kidney and bladder) and reproductive tract (uterus, uterine tube and ovary) using the Dneasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's recommendations. The polymerase chain reaction (PCR) was carried out according to Pimenta et al. (2019) with the primers *LipL*32-45F (5'-AAGCATTACCGCTTGTGG-3') and *LipL*32-286R (5'-GAACTCCCATTTCAGCGATT-3'), described by Stoddard et al. (2009), to amplify *LipL*32 gene, which is specific for pathogenic leptospires. The Kennewick serovar of the Pomona serogroup was used as a positive control, and ultrapure water as a negative control.

Sequencing reactions were carried out with 16S rRNA gene primers using the Big Dye Terminator v3.1 Kit (Applied Biosystems, Foster City/CA, USA). A 3130xL genetic analyzer and POP-7 polymer (Applied Biosystems, Foster City/CA, USA) were used for capillary electrophoresis (Platt et al. 2007). The sequence was aligned using BioEdit (Hall 1999), and compared with *Leptospira* strains obtained from Genbank (National Biotechnology Information Center, Bethesda/MD, USA)[5], using the BLAST tool[6]. The phylogenetic tree was explored in the Seaview4 software (Gouy et al. 2010), generated by the PHyML method using the GTR model, bootstrap value of 1,000 repetitions[7] visualized using FigTree v1.4.3[8]. The phylogenetic reconstruction included leptospira sequences from Genbank.

Statistical analysis. The proportions of positive animals according to biological material were compared using the chi-square test with Yates continuity correction, using BioEstat 5.3 software (Ayres et al. 2007), considering a significance level of 5% ($P \leq 0.05$). The agreement between serological and molecular results according to biological samples was checked with the Kappa test using the DagStat software (Mackinnon 2000).

RESULTS

Of the 40 animals, 26 (65%) tested positive for *Leptospira* spp. in at least one of the diagnostic tests used, and 25 (62.5%) animals were positive in at least one PCR sample. MAT detected anti-*Leptospira* spp. antibodies in two (5%) pigs and the detected serogroups were Australis (one animal) and Bataviae (one animal) with titers of 25 and 50 (Table 1).

PCR identified *Leptospira* spp. DNA in 25 (62.5%) animals. Only one (2.5%) animal was positive at PCR and serology. Four of the 200 samples collected could not be analyzed because they were contaminated, making DNA extraction impossible. Of the 196 analyzed samples, 60 (31%) were positive in the different diagnostic methods (Table 1).

Molecular detection of *Leptospira* spp. was carried out on 156 of the 160 samples collected, of which 54 (34.6%) were positive, 16/39 (40%) from the reproductive tract, 13/39 (32.5%) from the urinary tract, 13/40 (32.5%) from vaginal fluid and 12/38 (30%) in urine samples. Of the 12 urine samples positive at PCR, only one was not positive in urinary tract tissues. Of the 13 animals positive at PCR of vaginal fluid, four (31%) had positive reactions in urine and of the 16 animals

[5] Available at <http://www.ncbi.nlm.nih.gov> Accessed on Aug. 14, 2023.

[6] Available at <http://www.ncbi.nlm.nih.gov/BLAST/> Accessed on Aug. 14, 2023.

[7] Available at <http://tree.bio.ed.ac.uk/software/figtree/> Accessed on Aug. 20, 2023.

[8] Available at <http://tree.bio.ed.ac.uk/> Accessed on Aug. 20, 2023.

Table 1. Pigs (n=28) slaughtered in the Caatinga biome, Brazilian semiarid, positive in at least one of the diagnostic tests (serology and PCR)

ID	PCR				MAT		
	Urine	Urinary tract	Vaginal fluid	Reproductive tract	Result	Serogroup	Titer
1	-	-	-	+	-	-	-
2	+	+	-	-	-	-	-
3	-	-	-	-	-	-	-
5	+	+	-	-	-	-	-
6	+	+	-	-	+	Bataviae	25
7	+	+	-	-	-	-	-
9	+	NA	+	+	-	-	-
10	+	+	+	+	-	-	-
11	NA	+	+	+	-	-	-
12	-	-	+	+	-	-	-
13	+	+	-	-	-	-	-
14	-	-	+	+	-	-	-
15	-	-	+	+	-	-	-
16	+	+	-	-	-	-	-
18	+	+	+	+	-	-	-
19	-	-	-	-	-	-	-
22	-	-	+	+	-	-	-
23	+	+	-	-	-	-	-
24	-	-	+	+	-	-	-
25	-	-	+	+	-	-	-
26	NA	-	-	+	-	-	-
27	+	+	-	-	-	-	-
28	-	-	+	+	-	-	-
30	-	+	-	-	-	-	-
33	+	+	+	+	-	-	-
36	-	-	-	NA	+	Australis	50
37	-	-	-	+	-	-	-
38	-	-	+	+	-	-	-

ID = animal identification, PCR = polymerase chain reaction, MAT = microscopic agglutination test, NA = not analyzed.

positive at PCR in the reproductive tract, 13 (81.25%) had positive reactions in vaginal fluid. The comparison between tissues and fluids from the reproductive and urinary tracts of sows indicated that there was no significant difference between the proportions (P>0.05) (Table 2). Genetic sequencing of one urine and one urinary tract tissue sample revealed 99% identity with *L. borgpetersenii* (Fig.1).

The agreement between serological and molecular results according to the biological samples is shown in Table 3. There was no agreement (Kappa <0) between PCR samples from the genital tract vs. urinary tract or serological results.

Table 2. Comparison among the tissues and fluids of the reproductive and urinary tracts of female pigs slaughtered in the Caatinga biome, Brazilian semiarid

Biological sample	Total number of animals	Animals positive at PCR (%)
Urine	38	12 (31.6)a
Urinary tract (tissues)	39	13 (33.3)a
Vaginal fluid	40	13 (32.5)a
Reproductive tract (tissues)	39	16 (41.03)a

PCR = polymerase chain reaction; [a] In the same column, equal lowercase letters indicate no significant difference between the proportions (P>0.05).

DISCUSSION

In this study carried out in the Caatinga biome, which is exclusive of Brazil, a cut-off point of 25 was used for serology, unlike the majority of seroepidemiological studies with pigs, in which the cut-off point adopted was 100 (Araújo et al. 2023). However, a cut-off point of 25 has been recommended for animals in the Brazilian semiarid region due to the unfavorable environmental conditions for the survival of leptospires, especially during dry periods (Santos et al. 2022). High temperatures can make it difficult to maintain *Leptospira* spp., resulting in a low frequency of animals with circulating antibodies (Soares et al. 2022). This study detected only 5% seroreactivity in pig serum samples collected during the dry season, reinforcing this statement.

The serogroups of *Leptospira* spp. found in this study were Australis and Bataviae. Antibodies against pathogenic serogroups of *Leptospira* spp. have been detected in French pig farms, with the Australis and Icterohaemorrhagiae serogroups identified among seropositive pigs (Naudet et al. 2022). In Italy, the Australis and Pomona serogroups have been reported as the most frequently detected in pigs (Tagliabue et al. 2016). The Australis and Bataviae serogroups are pathogenic to humans, and pig populations exposed to these agents

represent a potential cause of occupational diseases, especially for farmers and slaughterhouse employees (Mirambo et al. 2018, Alashraf et al. 2020). As there is no selective carrier of *Leptospira* spp. of the Australis serogroup described among commensal rodents, pigs themselves may be the main host and reservoir for this agent in the context of pig farming (Ellis 2015, Naudet et al. 2022). Results on the seroprevalence of anti-*Leptospira* spp. antibodies are fundamental for a better understanding of the epidemiology of infections caused by the pathogen since, on farms that do not adopt technical care and vaccination protocols, the detection of antibodies in the herd can be directly associated with infection (Santos et al. 2023).

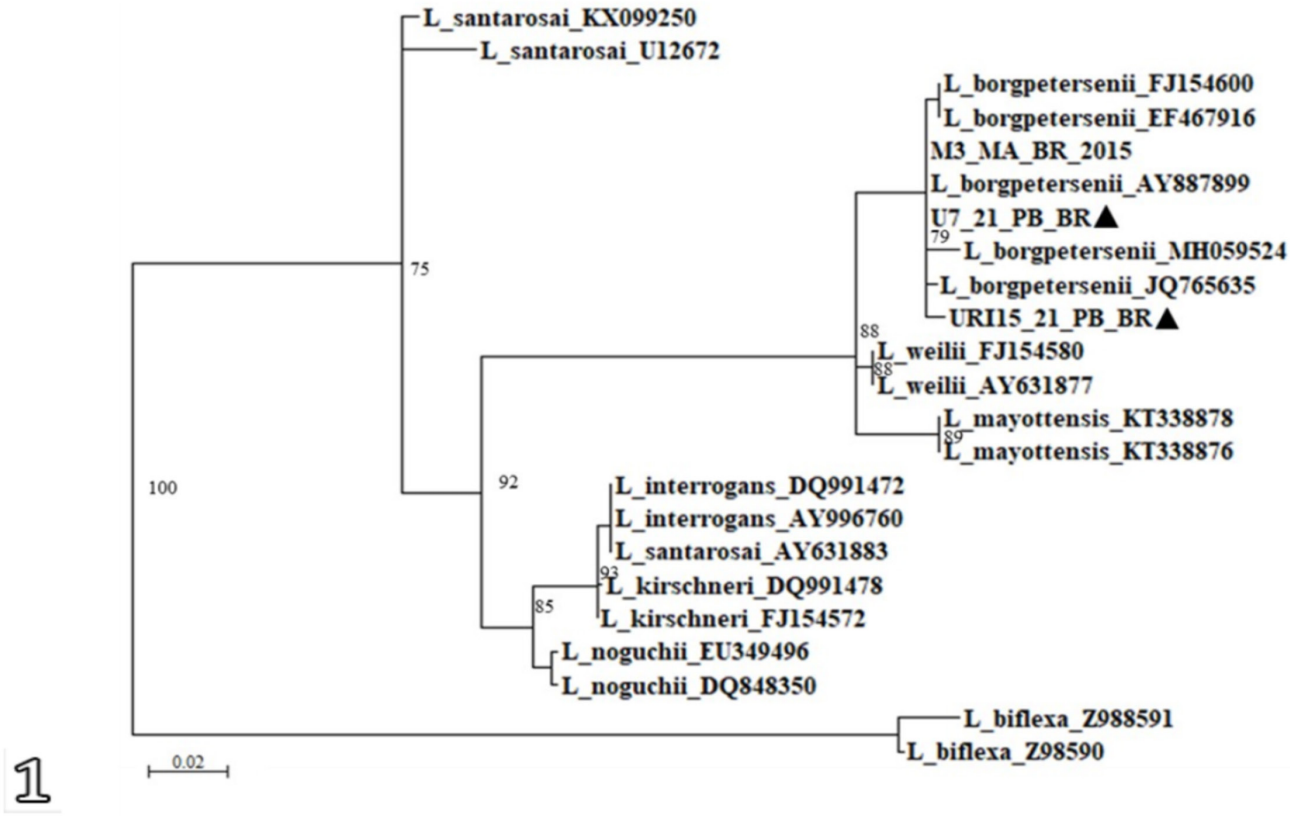

Fig.1. Phylogenetic tree based on the alignment of nucleotide sequences of the *LipL32* gene *Leptospira* sp., constructed using the neighbor-joining model with 1000 replicas. ▲ Sequenced samples.

Table 3. Kappa test applied to verify the agreement between serological and molecular results according to the biological samples

Biological samples	Results	Urine		Urinary tissues		Vaginal fluid		Serology (MAT)	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Urinary tissues	Positive	11 (44)	1 (4)	*	*	*	*	1 (3.7)	12 (44.4)
	Negative	0 (0)	13 (52)	*	*	*	*	1 (3.7)	13 (48.1)
	Kappa	0.92 (almost perfect)		*		*		0.006 (none)	
Vaginal fluid	Positive	4 (23.1)	8 (30.8)	4 (14.8)	8 (29.6)	*	*	0 (0)	13 (46.4)
	Negative	8 (30.8)	6 (23.1)	9 (33.3)	6 (22.2)	*	*	2 (7.1)	13 (46.4)
	Kappa	-0.238 (none)		-0.264 (none)		*		-0.141 (none)	
Reproductive tissues	Positive	4 (16)	10 (40)	4 (15.4)	11 (42.3)	13 (48.1)	3 (11.1)	0 (0)	16 (59.3)
	Negative	8 (32)	3 (12)	9 (34.6)	2 (7.7)	0 (0)	11 (40.7)	1 (3.7)	10 (37)
	Kappa	-0.433 (none)		-0.538 (none)		0.779 (moderate)		-0.075 (none)	
Serology (MAT)	Positive	1 (3.8)	1 (3.8)	*	*	*	*	*	*
	Negative	11 (42.3)	13 (50)	*	*	*	*	*	*
	Kappa	0.13 (none)		*		*		*	

MAT = microscopic agglutination test; Frequencies (%) were calculated regarding the total number of animals used in each comparison.

For the diagnosis of leptospirosis, the MAT technique is not suitable for identifying carrier animals, but it is a good screening tool and necessary to identify exposure to leptospires (Otaka et al. 2012). PCR-positive animals may not show seroreactivity at MAT, reiterating the benefit of PCR in detecting *Leptospira* spp. carriers (Lilenbaum et al. 2008, Almeida et al. 2019). In this study, 22 pigs tested positive at PCR and were negative at MAT. Moreover, 25 (62.5%) animals were positive in at least one PCR sample. These results highlight the importance of PCR to identify leptospire-carrying animals, which play an important role in the epidemiology of leptospirosis, remaining in the environment and transmitting the disease without clinical signs.

Leptospira spp. DNA was detected in 13 (32.5%) of the vaginal fluid samples. In this context, genital leptospirosis has been considered a specific syndrome for cattle with characteristics such as low antibody titers and chronic infection. In wild boars hunted in the Tuscany region (Italy), *L. fainei* has been detected in testicles and epididymis (Loureiro & Lilenbaum 2020, Cilia et al. 2021). The detection of leptospire DNA in the vaginal secretion of pigs suggests the possibility of the reproductive tract acting as an important extra-renal site of the disease. It, therefore, highlights the relevance of pigs as *Leptospira* spp. carriers, increasing the risk of infection for other pigs due to the close contact between animals and the occupational risk for humans (Fernandes et al. 2020).

In this study, of the 16 animals that tested positive for PCR in the reproductive tissues, 13 (81.25%) tested positive for PCR of vaginal fluid. Notably, there was no agreement (Kappa <0) between PCR samples from the genital tract vs. urinary tract or serological results. However, the agreement between reproductive tissues and vaginal fluid PCRs was moderate. The presence of leptospires in the vaginal fluid may be associated with uterine infection and has the potential to act as a shedding route for transmitting leptospirosis both during mating and swine reproduction (Ellis 2015, Loureiro & Lilenbaum 2020). The possibility of venereal transmission from males to females is well documented in cattle, and it has been proven that pathogenic *Leptospira* spp. in semen is able to colonize the reproductive tract and reach the uterus and oviduct. However, its role in transmission between wild and domestic pigs has not yet been established and is only a hypothesis (Loureiro & Lilenbaum 2020, Cilia et al. 2021). Although this research did not evaluate semen, the results are strong enough to suggest that female-to-male venereal infection in this species is possible and should be investigated.

Some studies on sheep suggest that genital infection is equal to or more important than kidney infection, especially in the dry season (Costa et al. 2018, Nogueira et al. 2020). These data reinforce the results found in this study, which identified high proportions of positive PCR results in the reproductive tract of pigs in the dry season, indicating a certain predilection of the bacteria for this system in periods with adverse environmental characteristics for its survival. In addition, when comparing the sensitivity and specificity of MAT, considering the PCR of the reproductive tract tissue pool as the gold standard, MAT showed greater sensitivity and specificity than PCR of other biological materials.

The two DNA samples sequenced from urine and urinary tract tissues showed 99% identity with *L. borgpetersenii*. This *Leptospira* species was detected in cattle (Allan et al. 2018), humans and *Rattus ratus* (Guernier et al. 2017). Molecular sequencing is an important tool for understanding the epidemiology of the disease, as it allows the identification of *Leptospira* species, generating results to understand how to prevent and intervene in the transmission of the disease (Lagadec et al. 2016, Fernandes et al. 2020).

The slaughterhouse is a place that can contribute significantly to the detection of specific pig diseases. The slaughterhouse can play an important epidemiological role in highlighting some zoonoses that are difficult to detect at the herd level. It is possible to state with certainty that the distribution of serogroups in pigs at the slaughterhouse represents the distribution of serovars that can be found on pig farms (Bertelloni et al. 2018). In addition, slaughterhouses are accessible locations for leptospire isolation studies, which is necessary and extremely important to characterize the circulating strains in the Caatinga and consequently use them as autochthonous antigens in serology and experimental infection studies.

CONCLUSIONS

Leptospirosis is a concern in pigs in the context of Caatinga, with a high prevalence of infection detected by different diagnostic methods.

Molecular analysis revealed a considerable proportion of infected animals.

The findings emphasize the importance of a multifaceted approach in the diagnosis of leptospirosis in pigs, with a focus on the use of genital tract samples for the diagnosis of leptospirosis in this animal species, providing valuable insights for the control and prevention of this disease in both animals and the zoonotic context.

Finally, the detection of leptospires in the genital tract indicates a possibility of male-female transmission in the venereal context.

Acknowledgments.- This study was supported by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), grant numbers 302222/2016-2 and 423836/2018-8, and "Fundação de Apoio à Pesquisa do Estado da Paraíba" (FAPESQ), grant numbers 46360.673.28686.05082021 and 54758.924.28686.25102022.

Conflict of interest statement.- The authors declare having no conflict of interest.

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