Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v44n3p1097

Prevalence and risk factors for *Leptospira* spp. in dairy cattle in western Paraná, Brazil

Prevalência e estudo dos fatores de risco da *Leptospira* spp. em bovinos leiteiros na região Oeste do Paraná, Brasil

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Highlights

Leptospira spp. infection is widely spread in the cattle population of western Paraná.
It is important to make the differential diagnosis.
The production system was one of the variables associated with infection in properties.
Serovar pomona Pomona and serovar australis Bratislava were the most prevalent.

Abstract

Leptospirosis is caused by spirochete bacteria of the genus *Leptospira* and is considered the most widespread zoonosis worldwide. It is an important agent that causes animal production to decrease. In cattle, it affects especially the reproductive tract. The objective of this study was to determine the seroprevalence of *Leptospira* spp., molecularly detect the bacteria in tissues of aborted fetuses, and identify the main risk factors associated with infection in cattle in dairy farms in Western Paraná. For this purpose, 600 bovine serum samples from 60 properties and 17 bovine fetuses from nine properties were collected. Data about the properties were also collected through an epidemiological questionnaire to assess the main risk factors associated with *Leptospira* spp. infection. The serum samples were analyzed using microscopic agglutination test (MAT), and the fetal tissues using nested polymerase chain reaction (nested PCR). Seroprevalence of *Leptospira* spp. in dairy cattle in Western Paraná was 39.83% (239/600) and none of the analyzed fetuses were positive for *Leptospira* spp. The main risk factors identified are related to the production system, reproductive management, and the presence of dogs on the property. *Leptospira* spp. infection is widely spread in the cattle population in Western Paraná.

Key words: Seroprevalence. Nested PCR. Leptospirosis. Cattle. Maintenance host.

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Received: June 05, 2022 Approved: Ago. 07, 2023

Resumo

A leptospirose tem como agente as bactérias espiroquetas do gênero *Leptospira* e é considerada a zoonose mais difundida pelo mundo. É um importante agente que causa diminuição da produção animal, sendo que nos bovinos ela acomete especialmente o trato reprodutivo. O objetivo desse estudo foi determinar a soroprevalência de *Leptospira* spp., detectar molecularmente a bactéria em tecidos de fetos abortados e identificar os principais fatores de risco associados à infecção em bovinos provenientes de propriedades leiteiras da região Oeste do Paraná. Para isso, foram coletadas 600 amostras de soro bovino provenientes de 60 propriedades e 17 fetos bovinos de nove propriedades. A partir de um questionário epidemiológico, também foram coletados dados das propriedades para avaliar os principais fatores de risco associados à infecção por *Leptospira* spp. As amostras de soro foram analisadas pela técnica de soroaglutinação microscópica (SAM) e os tecidos fetais por meio da Dupla Reação em Cadeia pela Polimerase (nested-PCR). A soroprevalência de *Leptospira* spp. em bovinos leiteiros da região Oeste do Paraná foi de 39,83% (239/600). Nenhum feto analisado apresentou resultado positivo para *Leptospira* spp. Os principais fatores de risco identificados estão relacionados ao perfil de produção, manejo reprodutivo e a presença de cães na propriedade. A infecção por *Leptospira* spp. está amplamente distribuída na população bovina da região Oeste do estado do Paraná.
Palavras-chave: Soroprevalência. Nested-PCR. Leptospirose. Bovinos. Hospedeiro de manutenção.

Introduction

Leptospirosis is a zoonosis caused by spirochete bacteria *Leptospira* spp. that infect several animal species, such as cattle, pigs, horses, dogs, as well as humans. Twenty-one genomic species have been described divided into pathogenic, intermediate, and saprophytic species and about 320 serovars, which belong mainly to the pathogenic species (Lehmann et al., 2014; Torres-Castro et al., 2017).

Cattle are considered to be maintenance hosts for the Hardjo serovar, as well as other members of serogroup *sejroe.* This serogroup is the most prevalent agent of *Leptospira* infection in cattle worldwide, in southern region this occurrence reaches 50% (Hashimoto et al., 2017; Barnabé et al., 2023). Others serovars related to infection in this animal species are *pomona* Pomona, *grippotyphosa* Grippotyphosa, *icterohaemorrhagiae* Icterohaemorrhagiae, *sejroe* Wolffi, and *canicola* Canicola (Lage et al., 2007; Mughini-Gras et al., 2014; Peregrine et al., 2006). The transmission happens through contact with urine or water contaminated with the bacteria, which enter the host organism through mucous membranes and the conjunctiva, small cuts, or even intact skin when there is pore dilation. After bacteremia, *Leptospira* spp. migrates to the renal tubules, where it remains and is eliminated in the animal's urine over a long period (Adler & Moctezuma, 2010).

The disease can affect cattle in two forms: the first is the acute form, in which animals have hyperthermia, hematuria, and jaundice; the second is the chronic form, in which animals have abortions, stillbirths, and estrus repetition. The last one is the most prevalent and brings the main losses to production (Adler & Moctezuma, 2010; Ellis, 1994).

The diagnosis of leptospirosis can be made using several techniques, such as immunohistochemistry, dark-field microscopy, serological techniques (e.g., enzyme-linked immunosorbent assay), complement fixation reaction, immunofluorescence, among others. However, to confirm the diagnosis and obtain information about the serogroups and serovars involved in the infections, culture, polymerase chain reaction (PCR), or microscopic agglutination test (MAT) are recommended. Since culture requires a long incubation period, i.e., it does not allow rapid diagnosis (Picardeau, 2013), PCR and MAT have been the most commonly used techniques. They are also the recommended techniques by the World Organization for Animal Health (World Organization for Animal Health [OIE], 2021).

The prevalence of leptospirosis in Brazil varies according to the region studied, the test used, and the risk factors (Hashimoto et al., 2012; Sarmento et al., 2012; Silva et al., 2012). Studies on the prevalence of this disease are essential since it is considered the most widespread zoonosis worldwide (Pinto et al., 2017). Thus, the objective of this study is to investigate the prevalence of leptospirosis in dairy cattle in Western Paraná using PCR and MAT, as well as correlating the results with an epidemiological questionnaire to identify the main risk factors associated with the disease.

Material and Methods

Ethics committee on animal use

This study followed the Ethical Principles of Animal Experimentation and was approved by the Ethics Committee on Animal Use of the Federal University of Paraná (UFPR, Setor Palotina, protocol number 50/2014).

Study area and sampling

The area chosen for the study on the prevalence of *Leptospira* spp. was the Western Region of the state of Paraná, since it is the main dairy basin of the state, with the cities of Marechal Cândido Rondon, Toledo, and Cascavel as the largest producers in the region (Instituto Brasileiro de Geografia e Estatística [IBGE], 2016).

To define the sample size, we performed the sampling in Epi Info (version 7.2.0.1). The estimated prevalence was 35%, following other studies conducted in the region (Hashimoto et al., 2012), the maximum expected error 5%, 95% confidence interval, and DEFF 1.5, which should be used when there is no random sampling, decreasing the error. The minimum number of samples to be collected was 525, but 600 animals were used, distributed in four cities of Western Paraná: Cascavel, Marechal Cândido Rondon, Palotina, and Toledo.

The number of cattle farms in each city was provided by the Paraná Agribusiness Defense Agency (*Agência de Defesa Agropecuária do Paraná* [ADAPAR]). We then calculated the properties from which the samples should be collected with proportional distribution (Table 1). The properties in each city were randomly selected, ranging from small-scale to large-scale production. We stipulated that samples would be collected from 10 animals on each property, totaling 60 properties.

Table 1
Distribution of properties and animals for sample collection in different cities in the Western Region of the state of Paraná, Brazil

City	Total number of properties	Sampled properties	Sampled animals
Cascavel	1,902	18	180
Toledo	2,000	19	190
M. C. Rondon	1,862	17	170
Palotina	591	6	60
Total	6,355	60	600

Sample collection and application of the epidemiological questionnaire

Blood samples were collected from the caudal veins of 600 cows with 40x1.2mm needles and 10mL syringes. After collection, the blood was stored in properly identified test tubes kept refrigerated until processing. Information such as the age and abortion history of each sampled animal were obtained applying an epidemiological questionnaire, which included information about the property, such as herd size, reproductive management, abortion history, among others. In the laboratory, the samples were centrifuged at 1500rpm for 10 minutes and then the sera were stored in microtubes at -20ºC until processing. Seventeen fetuses from nine properties were also collected: five located in Palotina, two in Cascavel, one in Toledo, and one in Marechal Cândido Rondon. Their kidneys, livers, and placentas were collected and stored in microtubes at -20ºC.

Sample analysis

Microscopic agglutination test (MAT)

For serological analysis, the samples were sent to the Leptospirosis Laboratory at the State University of Londrina (Universidade Estadual de Londrina), where they were tested using the microscopic agglutination test (MAT) for antibodies against 10 serogroups (australis, autumnalis, ballum, canicola, grippotyphosa, icterohaemorrhagiae, pomona, pyrogenes, sejroe and tarassovi) and 12 serovars (Bratislava, Butembo, Castellonis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Copenhageni, Pomona, Pyrogenes, Hardjo, Wolffi, and Tarassovi). The serum samples were screened at a 1:100 ratio. It is important to highlight that some serovars may present cross-reactions with others. This situation may occur within the same serogroup, that is, in the samples analysed in this study there may be cross reaction between Icterohaemorrhagiae and Copenhageni, and between Hardjo and Wolffi.

Polymerase chain reaction (PCR)

To analyze the fetuses, nested polymerase chain reaction (nested PCR) was performed at the Biotechnology Laboratory of the Federal University of Paraná - Setor Palotina. For this, the collected organs (kidney, liver, and placenta) were individually macerated and submitted to DNA extraction by the CTAB method, described by Chiriboga et al. (2015), and using the commercial DNeasy Blood and Tissue kit (Qiagen).

For CTAB, organ samples were macerated and centrifuged. The pellet was resuspended in a 700µl CTAB lysis buffer (100mM Tris-Cl, pH 8.0, 714mM NaCl, 20mM EDTA, 2% CTAB (Cetrimonium Bromide)), incubated at 65°C for two hours and shaken every 15 minutes. Subsequently, 700µL of Chloroform - Isoamyl alcohol (24:1) were added to the samples, which were then centrifuged at 12000 rpm for five minutes. The supernatant was placed in another microtube and 1000µL of absolute ethanol were added, staying overnight at -20ºC. Then, the contents were centrifuged at 14000 rpm for 15 minutes, the pellet resuspended in 1000µl of 70% ethanol, and centrifuged at 14000 rpm for 15 minutes. The supernatant was discarded and the pellet left to dry at room temperature. Finally, the pellet was resuspended in 50µL of TAE buffer (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA) (Chiriboga et al., 2015).

DNA extraction using the commercial DNeasy Blood and Tissue kit (Qiagen) was performed according to the manufacturer's recommendations.

To perform the nested PCR, the primers used were A, 5'-GGCGGCGCGTCTTAAACATG-3'; B, 5'-TTCCCCCCATTGAGCAAGATT-3'; C, 5'-CAAGTCAAGCGGAGTAGCAA-3'; and D, 5'-CTTAACCTGCTGCCTCCCGTA-3' (Mérien et al., 1992). The two reactions were performed with a final volume of 25µL, with a 10X buffer, 400µM DNTP, 1mM $MgCl_2$, 10µM of each primer, 1U Taq with initial denaturation at 94ºC-3 min, 29 cycles at 94ºC-1 min / 63ºC-1.5 min / 72ºC-2 min, and final extension at 72ºC-10 min. Amplified samples were submitted to agarose gel electrophoresis (1.5%) for visualization.

A commercial inactivated vaccine was used as a positive control. ultrapure water was used for the negative control. Both positive and negative controls were subjected to the stages of extraction (by the two methods), nested PCR and electrophoresis together with the samples.

Statistics

The results obtained and the data from the epidemiological questionnaire were analyzed in Epi Info (v. 7.2.0.1) through the chi-square tabulated in a 2x2 order and Yates correction, with the objective of evaluating the main risk factors related to leptospirosis in dairy cattle from rural properties in Western Paraná.

Results and Discussion

Seroprevalence

The prevalence of *Leptospira* spp. in the sample studied, based on the MAT, was 39.83% (239/600). Of the 60 properties analyzed in this study, 81.67% (49) had at least one seropositive animal (Table 2).

Table 2
Prevalence of anti-*Leptospira* spp. antibodies in dairy cattle in the Western Region of the state of Paraná, Brazil

City	Positive	Negative	%	Positive	Negative	%
Cascavel	56	124	31.11	15	3	83.33
Toledo	104	86	54.74	19	0	100
Marechal Cândido Rondon	74	96	43.53	13	4	76.47
Palotina	5	55	8.33	2	4	33.33
Total	239	361	39.83	49	11	81.67

In other studies, conducted in Brazil, the occurrence of *Leptospira* spp. varied from 6.44% to 98.8% depending on the state, number of animals evaluated, diagnostic method, and cutoff point used (Fávero et al., 2001, 2017; Pasqualotto et al., 2015). In a study conducted in the Western Region of the state of Santa Catarina, Fávero et al. (2017) found low seroprevalence of *Leptospira* spp. (6.44%) in animals with titers of 1:100 or more, while Pasqualotto et al. (2015) identified a seroprevalence of 31.67%, but using a smaller number of animals and properties with a lower level of technification. In this case, the discrepancy observed in the same region's seroprevalence rates can be justified by the number of samples analyzed and the level of technification of the properties studied. According to Fávero et al. (2017), the level of technification is directly related to the seroprevalence of *Leptospira* spp. in cattle herds.

A higher seroprevalence was observed in Paraná's bordering states, such as Mato Grosso do Sul, where rates reached up to 98%, which can be attributed to the region's climatic conditions that favor the maintenance and dissemination of the agent (Miashiro et al., 2018; Sarmento et al., 2012).

In dairy cattle in the South-Central Region of Paraná, Hashimoto et al. (2012) analyzed 1,880 samples using MAT (1:100) and found a seroprevalence of 36% for *Leptospira* spp., similar to the result found in the present study in Western Paraná. Similarly, in Southwestern Paraná, using MAT and the cutoff point of 1:100, Porto et al. (2018) found a seroprevalence of 39.28% for one or more *Leptospira* spp. serovars, also very similar to the result observed in the present study applying the same technique and cutoff point. This similarity regarding seroprevalence in Western and Southwestern Paraná may be explained by the animal trade between these regions, as well as by their geographical proximity.

The results obtained demonstrate that leptospirosis is widely spread in the studied region, since seropositive herds were identified in all cities where collections were performed, similar to the results of studies by Fávero et al. (2001) and Miashiro et al. (2018). All properties located in Toledo were positive, while the cities of Cascavel,

Marechal Cândido Rondon, and Palotina showed positive results in 73% of their properties.

A study carried out by Fávero et al. (2001) analyzed properties in 21 Brazilian states; in the state of Paraná, 83.3% of the studied properties presented at least one seropositive animal. In the other states, the percentages ranged from 74% to 100%. The state with the highest prevalence was Mato Grosso do Sul, with 100% of its properties presenting at least one seropositive animal.

In cattle in the state of Paraíba, Northeastern Brazil, Barnabé et al. (2023) using MAT with cutoff point of 1:50 analyzed 42 samples and found a seroprevalence of 64,3%. In this case, Sejroe, Tarassovi, Australis, Ballum, Djasiman and Hebdomadis were the reactive serogroups. Sejroe was the most prevalent serogroup (58,17%). Similar to the results of serogroup was observed by Gonçalves et al. (2021), but with lower sero prevalence. In a border region of Brazil with Paraguay, the authors analyzed 70 samples using MAT and found a seroprevalence of 42.86% for *Leptospira* spp. They believe that the pathogen that causes this disease may have its entry in Brazilian herds facilitated by the existence of a large extension of land borders.

Studies show that the most prevalent serogroups in cattle are *sejroe* and *pomona* (Silva et al., 2012; Fávero et al., 2001; Miashiro et al., 2018). In the present study, we found animals positive for nine serogroups (Figure 1).

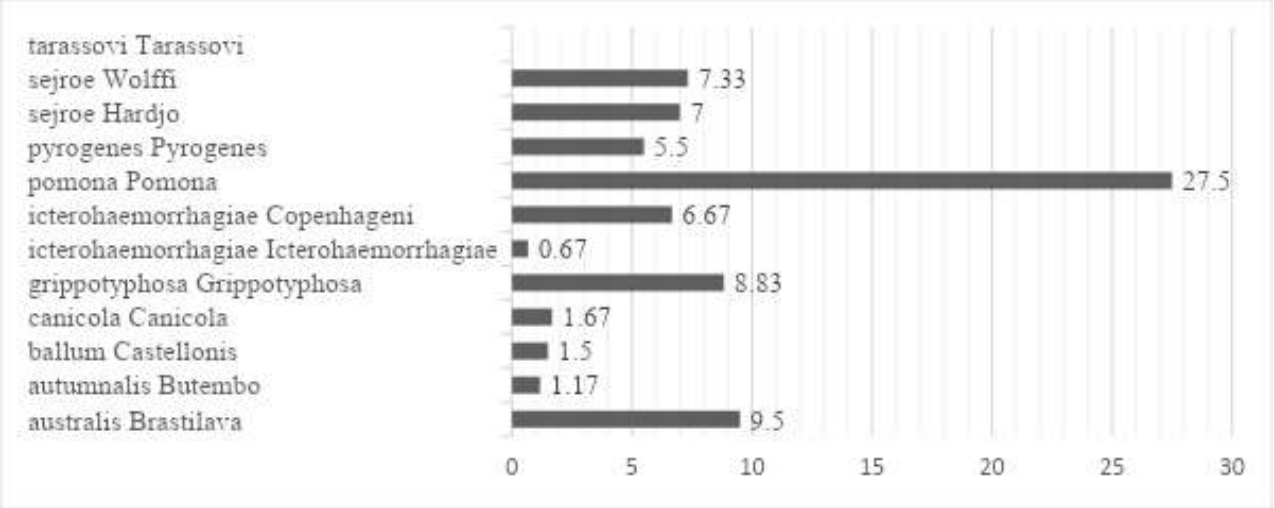

Figure 1. *Leptospira interrogans* serovars found in animals of 60 properties located in different cities in the Western Region of the state of Paraná, Brazil.

The *pomona* serovar Pomona (27.5%) was the most predominant in the total number of animals evaluated, as also found in a study by Fávero et al. (2017). According to Suepaul et al. (2011), the infection of cattle by the serogroup *pomona* may be linked to contact with animals of other species, such as pigs, sheep, and goats, which are disease reservoirs. The serogroups *australis* and *grippotyphosa* showed a seroprevalence of 9.5% and 8.8%, respectively. According to Ellis (2015), these serogroups are among those causing accidental infections in herds in some parts of the world, as are serogroups Autumnalis, Canicola, Hebdomadis, Icterohaemorrhagiae, Javanica, Pyrogenes, Sejroe, and Tarassovi. The maintenance of the serogroup grippotyphosa may occur due to the presence of several species of small rodents in the region, which is also associated with its occurrence in dogs, since they share their environment (Desvars et al., 2013).

In the present study, none of the animals presented antibodies against the *tarassovi* serovar Tarassovi. The other serovars and their respective seroprevalence were: *sejroe* Wolffi and sejroe Hardjo (7.3% and 7%), *icterohaemorrhagiae* Copenhageni (6.7%), *pyrogenes* Pyrogenes (5.5%), *canicola* Canicola (1.7%), *autumnalis* Butembo (1.2%), and *icterohaemorrhagiae* Icterohaemorrhagiae (0.7%).

The occurrence of the serogroups *sejroe* and *pomona* suggests that animals present an active immune response to *Leptospira* spp. In addition, a high frequency of these serovars has been found in animals from different dairy basins (Paixão et al., 2016). According to Langoni et al. (1999), dairy herds have 1.9 times more seroreactive animals when compared to those intended for beef production.

The serogroup *icterohaemorrhagiae* have synanthropic rodents as their main maintenance hosts. These animals are resistant to the clinical disease, acting as asymptomatic carriers and contaminating utensils used in animal handling as well as food and water through the elimination of bacteria in their urine (Paes et al., 2016).

Aborted bovine fetuses

During the course of this study, 17 bovine fetuses were collected from dairy herds of nine properties in the studied region: four from Cascavel, one from Marechal Cândido Rondon, two from Toledo, and 10 from Palotina. Kidney samples of all 17 fetuses were analyzed, but only three placenta samples due to the difficulty in collecting the material. All fetuses tested negative for *Leptospira* spp. by the nested PCR technique using both DNA extractions, similar to what was observed by Gagnon et al. (2010) when analyzing placenta and tissue samples of aborted fetuses in Quebec, Canada.

The observed data differ from the percentage of positive cases (6.2%) found by Genovez et al. (1993) who, using bacterial isolation and/or indirect immunofluorescence, analyzed 257 bovine fetuses from six Brazilian states, with *Leptospira* spp. as one of the most frequent bacterial agents in their study. Using polymerase chain reaction

(PCR), Cortez et al. (2006) found a prevalence of 3.2% for *Leptospira* spp. after analyzing samples from 114 aborted bovine fetuses and 10 calves with perinatal death.

The results obtained in the present study can be justified by the high positivity of the same samples when the protozoan *Neospora caninum* was investigated (52.94%), using the PCR technique (Snak et al., 2018), as well as the increased vaccination (Balamurugan et al., 2018) or the fact that infection can occur early in gestation, leading to embryonic losses and a return to estrus (Ellis, 1994). It is worth noting that all analyzed fetuses were between the fourth and eighth gestational months. In addition, the number of collected fetuses was lower than other studies that obtained positive results.

Analysis of factors associated with Leptospira spp. infection

The risk factors associated with *Leptospira* spp. infection in dairy cattle in the Western Region of the state of Paraná (Table 3) include the production system: properties that use the intensive production system are 1.94 times more likely to present seropositivity for *Leptospira* spp. than other properties; reproductive management: properties that use of Artificial Insemination (AI) and Fixed-Time Artificial Insemination (FTAI) techniques are 2.7 times more likely to present seropositive animals than those that use another type of reproductive management, such as the use of bulls jointly with AI; milk production: properties that produce above 1000 liters/day are 3.48 times more likely to present seropositive animals than properties that produce up to 200 liters/day, 200 to 500 liters/day, or 500 to 1000 liters/day; properties that acquire animals above two years of age for herd replacement are 2.05 times more likely to present seropositivity when compared to those that do not replace their herd with external animals; presence and number of dogs on the property: properties that have dogs are 2.76 times more likely to present seropositive animals. If the number of dogs is between 8 to 10, they are 2.14 times more likely to present seropositive cattle than properties that have 1 to 7 dogs; cattle that have contact with dogs are 1.63 times more likely to be seropositive for *Leptospira* spp.

The main variables related to infection were the production system, reproductive management, and the presence of dogs on the property. Intensive production refers to the use of techniques that aim to increase production, among which are AI and/or FTAI for reproductive management. In this study, this type of production as well as the use of artificial insemination techniques and high daily milk production (>1000L) were considered as important risk factors ($p = <0.001$). Cattle raised in intensive production are usually kept in more crowded environments, which provides proper conditions for the maintenance of *Leptospira* and, consequently, the animals become more exposed to the agent and more likely to be infected. Thus, leptospirosis can be associated with animal density rate (Paixão et al., 2016). According to Thrusfield (2007), as the density rate and exposure time increase, the spread of diseases is facilitated within populations.

Table 3
Risk factors related to the presence of anti-*Leptospira* spp. antibodies in dairy cattle from farms in the Western Region of the state of Paraná, Brazil

Variables		OR	p	
Property				
	Extensive	0.64 (0.43-0.95)		0.033
Production system	Semi-intensive	ns		0.529
	Intensive	1.94 (1.3-2.92)		0.001
	Bull	0.53 (0.36-0.77)		0.001
	FTAI	ns		0.128
Reproductive management	AI	ns		0.291
	FTAI and AI	2.7 (1.81-4.03)	<0.001	<0.001
	AI and bull	ns		0.91
	AI, FTAI, and bull	2.36 (1.11-5.01)		0.03
	1-100	ns		0.186
	100-200	0.27 (0.14-0.52)	<0.001	<0.001
Milk production (liters)	200-500	ns		0.22
	500-1000	ns		1
	>1000	3.48 (2.26-5.37)	<0.001	<0.001
	No	ns		0.74
	1 year	0.41 (0.27-0.62)	<0.001	<0.001
Animal replacement	2 years	1.81 (1.14-2.85)		0.013
	> 2 years	2.05 (1.2-3.5)		0.011
Dogs on the property		2.76 (1.11-6.87)		0.037
	0	0.36 (0.14-0.89)		0.037
Nº dogs	1-3	ns		0.5
	4-7	ns		0.252
	8-10	2.14 (1.18-3.88)		0.015
Abortion cases		ns		0.41
Individual/Animal				
Abortion history		ns		0.344
Contact with dogs		1.63 (1.04-2.55)		0.038

Legend: ns = non-significant.

In the process of natural mating, the semen is deposited in the female's vagina, where nonspecific defenses end up hindering the development of infections. However, in AI, semen is deposited directly into the uterus, which facilitates infection. Paixão et al. (2016) identified the use of AI as a protective factor in their study, but reinforced the existence of a relative sensitivity of *Leptospira* spp. to freezing and to antimicrobials used in semen preservation and that the semen should be submitted to a strict quality control in the centers, from collection to the preparation of the doses. Another important factor for the application of reproductive biotechniques is the need for previous training for their adequate execution.

In this study, the presence of dogs on the property was also considered a risk factor for leptospirosis (p=0.037), especially when in large numbers (8-10 dogs) (p=0.01). Fávero et al. (2017) observed that cattle are more prone to seropositivity in properties where dogs have access to pasture. Other authors have shown that the presence of dogs is epidemiologically relevant, especially in the maintenance of the *sejroe, icterohaemorrhagiae* and *canicola* serogroups (Balamurugan et al., 2018; Chiebao et al., 2015; Pinto et al., 2017; Suepaul et al., 2011). The serogroup *canicola* is the most identified in dogs, which are considered its maintenance hosts. However, these animals have been pointed out as incidental hosts of other serovars, such as Bratislava, which is reported to cause the clinical disease in this species (Paes et al., 2016) and was the second most prevalent serovar (9.5%) in the present study.

Animal replacement was identified as another risk factor for infection, especially the purchase of animals older than two years (OR=2.05). The acquisition of external animals without adequate sanitary control can facilitate the introduction of agents such as *Leptospira* spp. on the properties (Hashimoto et al., 2012). Paixão et al. (2016) observed that the frequency of *Leptospira* spp. serovars by municipalities was influenced according to the sanitary management adopted by the properties. In this case, the Hardjo and Pomona serovars were not detected in 38.46% (5/13) of the analyzed municipalities. Avoiding the insertion of animals carrying serovars into the herd, consequently, nullifies the chance of access to contaminated environments. The other factors analyzed, such as abortion history and cases, among others, showed no significant differences.

Extensive production system, reproductive management through the use of bulls, low milk production (<200 liters/day), herd replacement by acquiring animals less than one year of age, and the absence of dogs on the property were identified as protective factors. These are the main factors found in small properties with a reduced number of animals; in most of these properties, the animals are kept in large environments, with reduced crowding and less direct contact between them, making the transmission of the disease difficult. Also, small properties generally have a lower turnover, i.e., less purchase of animals. In this case, the replacement is normally done by the property's own animals, which justifies this style of property presenting lower rates of leptospirosis.

Conclusion

Leptospira spp. infection is widely spread in the cattle population in Western Paraná. Although the *pomona* serovar Pomona and *australis* serovar Bratislava are the most prevalent in this study, they are not the only ones circulating in the cattle population in that region, which should be considered for the application of prophylaxis and control practices for this disease. In addition, the presence of dogs on the property, the production system, and reproductive management of cattle were the main variables related to infection in the studied properties. In contrast, *Leptospira* spp. DNA was not identified in the samples of aborted fetuses used in this study, which highlights the importance of performing differential diagnosis for the various agents affecting the reproductive tract of cattle.

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