Scientific Article

Medicina Veterinária

e-ISSN 2675-6617
v. 18 n. 2 (2024)

Leptospira spp. in dairy cattle in a family farming system

Leptospira spp. em bovinos leiteiros criados em sistema de agricultura familiar

Vera Cláudia Magalhães **Curci**[1], Fernanda Senter **Magajevski**[2], Fernando Christiano Gabriel **Moreli**[3], Adriana Hellmeister de Campos Nogueira **Romaldini**[4], Ricardo Lopes Dias da **Costa**[5*], Talita Carolina Bragança de **Oliveira**[6], Tereza Cristina **Cardoso**[6], Suzane **Manzini**[7], Maria Eduarda **Cavalheiro**[7], Marcela **Alexandrino**[7], Thainá Valente **Bertozzo**[7], Jackieline Sampaio **Steinle**[8], Andresa Xavier Frade **Gomes**[8], Isabella Neves **Aires**[9], Simone Baldini **Lucheis**[10], Raul José Silva **Girio**[11]

[1]*Instituto Biológico, Agência Paulista dos Agronegócios (SAA-SP), Araçatuba-SP, Brazil.*
[2]*Prefeitura Municipal de Araras, Centro de Reabilitação de Animais Silvestres, Araras-SP, Brazil.*
[3]*Fundação do Instituto de Terras do estado de São Paulo, Andradina-SP, Brazil.*
[4]*Instituto Biológico, Agência Paulista dos Agronegócios (SAA-SP), São Paulo-SP, Brazil.*
[5]*Instituto de Zootecnia, Agência Paulista dos Agronegócios (SAA-SP), Nova Odessa-SP, Brazil.*
[6]*Faculdade de Medicina Veterinária, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus de Araçatuba, Araçatuba-SP, Brazil.*
[7]*Faculdade de Medicina de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus de Botucatu, Botucatu-SP, Brazil.*
[8]*Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus de Botucatu, Botucatu-SP, Brazil.*
[9]*Faculdade de Ciências, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus de Bauru, Bauru-SP, Brazil.*
[10]*Agência Paulista de Tecnologia dos Agronegócios, Bauru-SP, Brazil.*
[11]*Curso de Medicina Veterinária, Universidade de Marília (UNIMAR), Marília-SP, Brazil.*
**Corresponding author: rldcosta@sp.gov.br*

Article Info

Keywords:
Antibodies
Zoonosis
Leptospirosis
Diagnosis

DOI
10.26605/medvet-v18n2-6404

Citação
Curci, V. C. M., Magajevski, F. S., Moreli, F. C. G., Romaldini, A. H. C. N., Costa, R. L. D., Oliveira, T. C. B., Cardoso, T. C., Manzini, S., Cavalheiro, M. E., Alexandrino, M., Bertozzo, T. V., Steinle, J. S., Gomes, A. X. F., Aires, I. N., Lucheis, S. B., & Girio, R. J. S. (2024). *Leptospira* spp. in dairy cattle in a family farming system. Medicina Veterinária, *18*(2), 159-168. https://doi.org/10.26605/medvet-v18n2-6404

Received: November 1, 2024
Accepted: April 3, 2024

Abstract

Leptospirosis is an endemic zoonosis in Brazil responsible for numerous reproductive and economic losses to the dairy industry. Brazil ranks fifth in global production of cow's milk, by and around 23 million cows are milked every day. The northwest region of the state of São Paulo has a large number of family farming settlements, with dairy farming being predominant. This article reports a seroepidemiological study to detect agglutinins against *Leptospira* spp. in 1,004 dairy cattle kept on 64 family farms producing milk in the northwest region of São Paulo. Management and environmental data for situational diagnosis were obtained through a questionnaire. Antibodies against *Leptospira* spp. were investigated by the microscopic agglutination test (MAT) and milk and urine samples were obtained from seroreactive animals with titer ≥400. These samples were submitted to molecular tests. Of the 64 farms studied, 63 (98%) had seroreactive animals. Of the 1,004 animals, 523 (52%) were reactive. According to the molecular tests, the presence of *Leptospira* spp. in the urine and milk of the seroreactive animals was negative. Presence of wetlands, rodents, dogs, cats and horses and occurrence of abortion were variables associated with the risk of infection by *Leptospira* spp. In addition to its importance in animal health, leptospirosis poses a risk to human health, a situation that indicates the need for improvement of sanitary conditions in small farms.

Resumo

A leptospirose é uma zoonose endêmica no Brasil responsável por inúmeras perdas reprodutivas e econômicas para a indústria de laticínios. O Brasil encontra-se na quinta posição na produção mundial de leite de vaca e cerca de 23 milhões de vacas são ordenhadas todos os dias. A região noroeste do estado de São Paulo possui considerável número de assentamentos trabalhando no sistema da agricultura familiar, sendo predominante a pecuária de leite. Objetivou-se realizar um estudo soroepidemiológico para detecção de aglutininas contra *Leptospira* spp. em 1.004 bovinos leiteiros criados em 64 propriedades produtoras de leite de agricultura familiar

na região noroeste do estado de São Paulo. Dados de manejo e do ambiente para o diagnóstico de situação foram registrados por meio de questionário. Anticorpos contra *Leptospira* spp. foram investigados pela prova de soroaglutinação microscópica (SAM) e amostras de leite e urina obtidas dos animais sororreagentes com título ≥ 400 foram submetidas às provas moleculares. Das 64 propriedades estudadas, 63 (98%) apresentaram animais sororreagentes. Dos 1.004 animais, 523 (52%) foram reagentes. Para as provas moleculares, a presença de DNA de *Leptospira* spp. na urina e no leite dos animais reagentes à sorologia foram negativas. Áreas alagadiças, presença de roedores, ocorrência de aborto, presença de cães, presença de gatos e presença de equinos foram variáveis que estavam associadas ao risco de infecção por *Leptospira* spp. Além da importância na saúde animal, a leptospirose acarreta risco à saúde humana, condição que serve de alerta para a melhoria das condições sanitárias nas propriedades rurais.
Palavras-chave: Anticorpos; zoonose; leptospirose; diagnóstico.

1 | Introduction

Brazil has 23 million cows milked every day, and milk production is present in 99% of rural properties, placing the country in the fifth position in the global production of bovine milk (Favero et al., 2017). The southeast region of Brazil occupies second place in the national ranking of milk production, that region's state of Minas Gerais being the largest Brazilian producer and the state of São Paulo occupying the 6th national position (IBGE, 2024).

Family farms are increasingly important for the production crops and rearing of livestock, producing a large part of the food consumed by the public in Brazil. Hence, there is a need to guarantee the quality of the products generated and the subsistence of farm families. Research related to the characterization of production systems and quality of products obtained, mainly from milk, has been carried out (Luna et al., 2020).

The northwest region of the state of São Paulo, close to the border with the state of Mato Grosso do Sul, has more than 3,500 family farming operations (Pietrafesa et al., 2018). Dairy farming, the main activity in the region, has attracted investments for the nutritional improvement of herds, in addition to research into the genetic parameters of dams and bulls. Besides self-consumption, the milk produced is sold directly to producers of dairy products or to cooperatives. Most families also adopt different forms of marketing, such as direct sales to consumers and/or retailing, including unprocessed or hand-processed products. Self-consumption of both milk and crops promotes the economic sustainability of farms (Sant'ana et al., 2007). The family production system is characterized by direct contact between humans and animals. This type of breeding requires care in relation to the transmission of zoonoses by water and food (Rahman et al., 2020).

Leptospirosis is a zoonotic infection caused by spirochete bacteria of the genus *Leptospira*, resulting from direct or indirect contact with contaminated urine, water or soil (Garba et al., 2017). It is responsible for numerous reproductive and economic losses in the dairy industry, since it causes reproductive problems such as abortions, infertility, stillbirths, retained placenta, and endometritis (Jain et al., 2019). It is particularly prevalent in poor areas, with high levels of social inequality, leading to serious economic and social losses (Martins and Spink, 2020). In Brazil, it is an endemic disease with epidemic peaks in the months of greatest rainfall (Diz and Conceição, 2021).

Cattle are recognized as maintenance hosts of Hardjo serovar as well as other members of the Sejroe serogroup, causing chronic disease with subclinical and persistent infection of the reproductive tract. Other serovars such as Pomona, Icterohaemorrhagiae, and Grippotyphosa have also been associated with bovine leptospirosis (Favero et al., 2017).

Domestic, wild, and synanthropic animals are reservoirs of leptospires, with the synanthropic rodent *Rattus norvegicus* (brown rat) being the main reservoir in rural and urban areas, thus posing the greatest risk of transmitting leptospirosis in those areas (Fornazari et al., 2018). Wild and synanthropic sources of infection such as the nutria (*Myocastor coypus*), coati (*Nasua nasua*), crab-eating fox (*Cerdocyon thous*), giant armadillo (*Euphractus sexcinctus*), skunk (*Didelphis* spp.), capuchin monkey (*Cebus apella*) and cavy (*Cavea aperea*), typically do not show signs of infection, hence acting as asymptomatic carriers by harboring leptospires in the kidneys and eliminating them in the environment (Oliveira et al., 2013).

Urine, fetus, or placenta from infected animals, post-abortion uterine discharges, and semen are the

most important sources of elimination of leptospires in herds (Clazer et al., 2017). Leptospires are usually present in the urine between the second and fifth week after infection, but convalescent animals may have leptospiruria for several months. In lactating animals, leptospires can be found in milk during the acute systemic phase of the disease. In fresh milk, they can survive for a few hours and in diluted milk for a few days (BRASIL, 2010).

Molecular biology techniques have been increasingly used for the diagnosis of leptospirosis in organic bodily fluids (Ferreira et al., 2021) such as bovine semen (Maiolino et al., 2021), fetuses from abortions (Dewes et al., 2020), urine (Manzini et al., 2021), and uterus, ovary and vaginal fluids from cows (Di Azevedo et al., 2021).

Important leptospirosis control measures are anti-rat actions, avoiding contact with flooded areas, isolation and treatment of sick animals, detection and treatment or sacrifice of asymptomatic carriers, and systematic immunization of animals. In order to avoid the presence of wild and synanthropic animals in the surroundings of rural properties, sanitary measures are needed, such as adequate disposal of garbage, adequate packaging of food and animal feed, and removal of debris, which can serve as shelter for reservoir animals (BRASIL, 2010).

A study of the prevalence of leptospirosis carried out in cattle from 21 Brazilian states, from 1984 to 1994, observed the presence of the serovar Hardjo in all the analyzed states, and that in the state of São Paulo, 79.9% of the farms evaluated had cases of the disease (Favero et al., 2001). The predominance of the Hardjo serovar in herds from southern and southeastern Brazil was also verified by Vasconcellos et al. (1997), and in Minas Gerais by Cosate et al. (2017).

Since there have been no studies of sanitary conditions in rural settlements in the northwest region of the state of São Paulo, and given the social impact of the disease, we carried out a seroepidemiological study of leptospirosis in dairy cattle kept by family farmers in a settlement in this region, to identify the predominant serovars, in order to guide the hygienic-sanitary measures through a process of awareness of the target community and actions such as treatment and vaccination of all animals of herds with indications of disease.

2 | Materials and Methods

The study was carried out in 64 milk-producing farms, with an average area of 16.14ha, located in a settlement in the municipality of Andradina, state of São Paulo. Among 2,014 cattle belonging to these families, 1,004 animals of reproductive age, of both sexes and different breeds, participated in the serological study to detect antibodies against *Leptospira* spp. Samples and information were collected from July to December 2010.

Management and environmental data for diagnosis of the situation were recorded through a questionnaire, consisting of open and closed questions, aimed at investigating clinical symptoms suggestive of the disease and the characteristics of the farms' local environments.

Antibodies against *Leptospira* spp. were investigated by the microscopic agglutination test (MAT) (Santa Rosa, 1970), with live cultures of leptospires in an enriched medium (EMJH, Difco®), using a microscope equipped with a dark field condenser (Axio Imager A.2 Carl Zeiss, Jena/Germany). The antigens used consisted of a collection of 24 standard strains of leptospires of the following serovars: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcomb, Cynopteri, Grippotyphosa, Hebdomadis, Copenhagen, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc, and Sentot.

Blood collections to test for antibodies against *Leptospira* spp. in cattle were carried out on different dates, with prior scheduling, according to the farmer's availability. All told, 1,004 samples were collected through venipuncture, using sterile disposable needles and Vaccutainer® test tubes and then kept at room temperature for one hour for desorption. To better organize the samples, a collection form was prepared for each property, in which data on the property and animals were recorded [date, name or number of property/lot, name of owner, registration (individual), breed, sex, and age of animals used for collection]. The samples were sent to the Regional Laboratory of Araçatuba of the Biology Institute for complete separation of the blood serum and clot samples, which were transferred to 2mL microtubes (duplicate), duly identified and kept in a freezer at a temperature of –20°C until the analysis (serological testing) was carried out. Sera were screened at 1:100 dilution, and

those showing 50% or greater agglutination were titrated by examining a two-ratio geometric dilution series. The serum titer was considered to be the reciprocal of the highest dilution that showed a positive result.

Animals can present variable titers such as 100IU or 200IU due only to contact with the agent, and sometimes the presence of antibodies can be a response to vaccination, Higher titers can indicate disease, and the animal can become a reservoir with the agent being eliminated through urine during the leptospiruria phase, contaminating the environment and infecting other animals and humans (Bharti et al., 2003). Thus, in an attempt to confirm the elimination of *Leptospira* in urine or milk, samples of those substances were obtained from seroreactive animals with titer ≥400 for leptospirosis in the MAT test (n=42). These samples were subjected to DNA extraction with the commercial reagent RTP Bacteria DNA Mini Kit (Invitek®), following the protocol for isolation of bacterial DNA from urine samples according to the manufacturer's recommendations. Sample volumes of 15mL (milk and urine) were centrifuged in Falcon tubes for 15 minutes at 2,200×g and the supernatant was discarded. The sediment formed at the bottom of each tube was resuspended with 3mL of 1x PBS and centrifuged for 5 minutes at 2,200×g. The supernatant was discarded again by inversion of the tube and 400µL of ressuspension buffer R contained in the extraction kit was added to the sediment. After homogenization by pipetting, the volume was transferred to Extraction L tubes previously identified with the analyzed samples. These tubes were incubated in an Eppendorf ThermoMixer® for 10 minutes at 65°C and then for 10 minutes at 95°C. Next, 400µL of Binding Buffer B6 was added to each sample and the tubes were vortexed. The volume was transferred to RTA Spin Filter Set tubes and incubated for 1 minute at room temperature. Then, it was centrifuged for 1 minute at 18,000×g and after discarding the filtrate, the filter was again coupled to the RTA Receiver Tube and 500µL of Wash Buffer I was added, followed by centrifugation at 18,000×g for 1 minute. The filtrate content was discarded and the filter was attached to a new RTA Receiver Tube. Subsequently, 600µL of Wash Buffer II was added and the tube was centrifuged for 1 minute at 18,000×g. The filtrate was again discarded, and the filter was attached to the tube for further centrifugation for 3 minutes at 30,000×g. The filter was then fitted into a 1,5mL receiver tube with further addition of 100µL of Elution Buffer D (pre-warmed) and incubated at room temperature for 1 minute. After centrifuging for 1 minute at 11,400×g, the filtrate was discarded and the material resulting from the process was identified and stored at -20°C until polymerase chain reaction (PCR) amplification.

For amplification of DNA from *Leptospira* spp., the genus-specific primers Lep1 (5' GGC GGC GCG TCT TAA ACA TG 3') and Lep2 (5' TTA GAA CGA GTT ACC CCC CTT 3') were used, as described by Mérien et al. (1992). Amplification of DNA samples was performed in 500µL microtubes, with a final volume of 45µL. The reaction mix consisted of 15µL of ultrapure water, 5µL of 10x reaction buffer (500mM KCl; 15mM MgCl2; 100mM tris-HCl, pH 9.0), 8µL of the dNTPs mixture (200mM of each nucleotide: dCTP, dATP, dGTP, dTTP), 1,5µL of 50mM MgCl2, 2,5 µL of each primer (10pMol/µL), 0,5µL of Taq DNA polymerase (5U/mL) and 10µL of the extracted DNA sample or ultrapure H2O for the negative control (Richtzenhain et al., 2002). The amplification in the thermocycler consisted of the following steps: denaturation of the strand at 94°C for 3 minutes, 35 cycles of DNA denaturation at 94°C for 60 seconds, annealing of the primers at 60°C for 60 seconds, and final extension at 72°C for 10 minutes.

The analysis of the amplified product (331 base pairs) was performed by electrophoresis in a 2.0% agarose gel + 12µL of ethidium bromide, diluted in 20mL of 5x TBE and 80mL of water. Then 10µL of a mixture containing 8µL of amplified product and 2 µL of dye (0.25% bromophenol blue, 30% glycerol) was placed in each of the wells of the gel. The gel was subjected to a constant voltage of 6 to 7V per centimeter of the distance between the electrodes in a horizontal bowl containing 1x TBE running buffer. After 1 hour, the development of the bands was observed in an ultraviolet transilluminator (300-320nm). The gel was photographed using a photodocumentation system (Kodak Digital DC Camera/120 Zoom) and analyzed with the 1D Image Analysis software (Kodak Digital Science).

A pure culture sample, serovar Hardjo strain Hardjoprajitino, was used as a positive control, and a PCR reaction mixture without DNA containing 10ml of ultrapure water was used as a negative control.

The serological data collected were entered into an Excel spreadsheet and presented as

frequencies per farm and serovar found. The data referring to the answers to the questionnaire were tabulated and stored using the Epi Info™ 7 program and presented as descriptive statistics

3 | Results and Discussion

Of the 64 farms studied, 63 (98%) had animals seroreactive for *Leptospira* spp. In several regions of Brazil, studies have indicated a high prevalence (70% to 100%) of herds reactive to *Leptospira* (Favero et al., 2001; Thompson et al., 2006; Lage et al., 2007; Castro et al., 2008; Fornazari et al., 2018). The percentage of farms with seroreactive animals in the settlement was alarming, so special attention should be given to bovine leptospirosis due to the threat to public health, production losses and environmental contamination (One Health).

Of the 1,004 bovine sera examined using the MAT test, 523 (52%) were reactive against one of the 24 serovars tested, a situation that can indicate previous contact with *Leptospira* spp.

Although the titers varied between 100 and 1,600, no clinical signs of the disease were observed in the cattle. The collection of samples was carried out in the first half of the year, when there were more favorable conditions of temperature and humidity in the northwest region of São Paulo, to the dissemination of leptospires, in addition to the abundant presence of wild fauna potentially acting as reservoirs. The MAT results revealed that the most frequent serovars found were Wolffi (27.1%), Grippotyphosa (24.0%), Hebdomadis (22.3%), Shermani (11.8%) and Autumnalis (11.2%) (Table 1 and Figure 1)

In Brazil, serological surveys carried out in cattle herds have revealed that the serovars Wolffi, Hardjo, Pomona, Grippotyphosa, Icterohaemorrhagiae, and Canicola are more frequent, with Hardjo serovar being the most prevalent (Favero et al., 2001; Thompson et al, 2006; Lage et al., 2007; Favero et al., 2017). However, some studies have reported the predominance of Wolffi serovar over the Hardjo serovar in reactive cattle, possibly reflecting greater occurrence of the former serovar in a given region (Ribeiro et al., 1999; Juliano et al., 2000).

The Grippotyphosa serovar has already been isolated from wild animals, dogs, swine, and horses (Pinheiro et al., 1974; Miller et al., 1990), and the Shermani serovar from swine and rodents (Oca et al., 1986). The serovars Hebdomadis and Autumnalis detected in cattle in the present study have also been observed in wild animals in the region studied on the border with Mato Grosso do Sul (Santa Rosa et al., 1980).

Attempts to detect the presence of *Leptospira* spp. in the urine and milk of the animals, following the extraction protocol of the commercial kit RTP Bacteria DNA Mini Kit (Invitek®), under the conditions in which the samples were collected, were negative (Figure 2). Although the animals showed low titers in serology and did not show clinical signs of the disease, convalescent animals can present leptospiruria for several months (BRASIL, 2010), with an intermittent character (Faine, 1982). In addition to the probability of samples without the presence of leptospires detectable by the test, the sensitivity of PCR can vary according to the chosen primers, biological material analyzed, conservation method, and sample storage time, since DNA can easily suffer degradation (Veloso et al., 2000). Negative urine PCR results can also occur due to the presence of inhibitors in the urine itself, such as urea, creatinine and acid radicals, in addition to factors such as acidic pH and freezing samples before proceeding with DNA extraction (Lucchesi et al., 2004).

In lactating animals, leptospires can be found in milk during the acute systemic phase of the disease (BRASIL, 2010). However, no animals were observed in this condition in the present study. Additionally, the possibility exists of collecting these materials for extraction five months after collection for serology.

Data acquired from the questionnaires applied to the 64 farmers indicated the existence of 257 people (206 adults and 51 children) residing in the settlement studied. It is supported by low-interest credit from the state government for implementation of farm plots, including money for housing construction and family maintenance in the first year (Buainain and Souza Filho, 1998). All farmers lived in brick houses with a structured sanitation system. The source of domestic water was 100% from a well and sewage was discharged in a septic tank, whose designs were prepared by Brazilian Company of Farming Research (Embrapa) and adopted by the Microbasin Program of Coordenadoria de Assistência Técnica Integral/Secretaria de Agricultura e Abastecimento do estado de São Paulo (CATI/SAA).

The management of household waste, owing to challenges to municipal collection, was executed by farmers through incineration (90.6%), interment (4.6%), or was left unprotected in the environment (4.8%).

Table 1. Titers of agglutinins of 523 dairy cattle reactive for leptospirosis by the microscopic agglutination test (MAT), and their respective serovars among 1,004 animals evaluated in a settlement, Andradina, state of São Paulo, 2010

Serovar	Titers					Total	% *
	100	200	400	800	1600		
Andamana	44	4	0	0	0	48	9.2
Australis	19	6	0	0	0	25	4.7
Autumnalis	45	12	2	0	0	59	11.2
Bataviae	3	0	0	0	0	3	0.5
Bratislava	28	0	1	0	0	29	5.5
Butembo	18	0	0	0	0	18	3.4
Canicola	38	2	0	0	0	40	7.6
Castellonis	32	3	0	0	0	35	6.6
Copenhageni	16	2	0	0	0	18	3.4
Cynopteri	8	0	0	0	0	8	1.5
Grippotyphosa	91	13	10	10	2	126	24.0
Hardjo	37	1	2	0	0	40	7.6
Hebdomadis	80	18	14	4	1	117	22.3
Icterohaemorrhagiae	2	0	1	0	0	3	0.5
Javanica	1	0	0	0	0	1	0.1
Patoc	23	1	0	0	0	24	4.5
Panama	0	0	1	0	0	1	0.1
Pomona	4	1	0	0	0	5	0.9
Pyrogenes	26	0	2	1	0	29	5.5
Sentot	16	3	0	0	0	19	3.6
Shermani	55	4	3	0	0	62	11.8
Tarassovi	12	3	1	2	0	18	3.4
Whitcombi	33	3	2	0	0	38	7.2
Wolffi	89	33	10	7	3	142	27.1

The production system consisted of dairy cattle (crossbred animals), living on the same property with animals of other species such as goats, pigs, horses, dogs, and cats. The animals were kept in a pasture system, but 17% of the farmers supplemented their animals with some type of feed, stored in sheds or drums. The animals' drinking water came from streams (32.8%) or a well (67.1%). According to the farmers, the cleaning of feed and water troughs was carried out regularly.

Reproduction was carried out exclusively by natural mating and 67.1% of the farms had a special

paddock for the end of gestation and parturition of the animals. The cattle were purchased from traders (35.9%) with a clearance certificate for brucellosis and tuberculosis, or from neighboring farmers (64.0%) without any health certificate. In addition, 28.1% of the respondents said they shared pastures with other farmers.

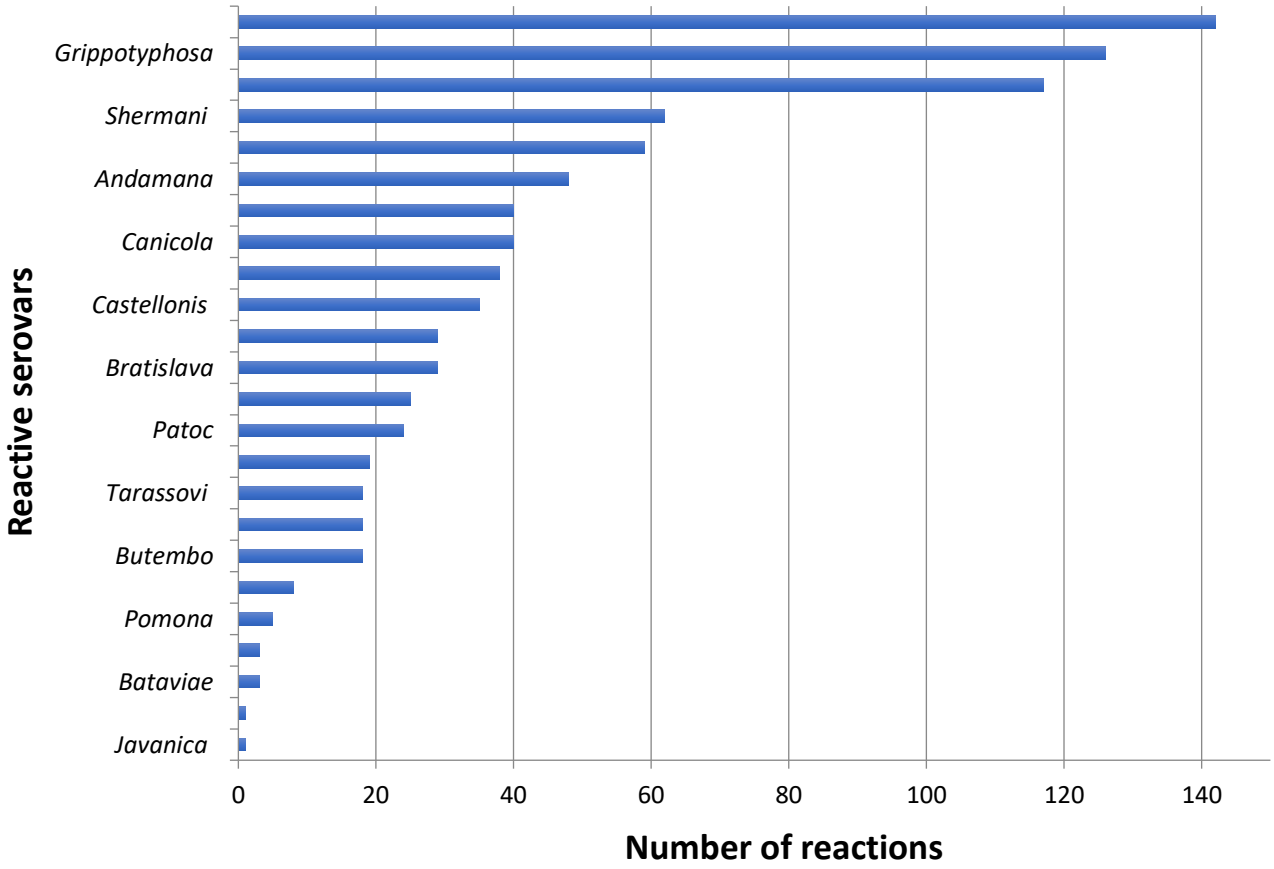

Figure 1. Graphic representation of *Leptospira* spp. serovars found in dairy cattle from Andradina, São Paulo state, considering all the reactive animals and their respective serological titers.

All properties had technical veterinary assistance from employees of the Land Institute Foundation of the State of São Paulo (ITESP), who also administered vaccinations against foot-and-mouth disease and brucellosis. Vaccination against symptomatic anthrax was performed in 92.1% of the animals by the farmers themselves, but vaccination against bovine leptospirosis was not performed.

There were no animals with clinical signs of bovine leptospirosis, but 34.3% of the farmers reported cases of abortions on the properties.

Regarding the presence of free-living wild animals, 84.3% of the farmers admitted the presence, mainly of rodents, with 73.4% claiming they carried out control measures. Of the 64 farms visited, 48.4% had wetlands with free access by the animals.

Variables possibly associated with the occurrence of antibodies against *Leptospira* spp., such as history of abortion, swampy areas on the property, purchase of animals without a sanitary certificate, presence of rodents and other free-living wild animals on the property, lack of adequate feed storage, contact with other domestic animals, shared pastures and lack of vaccination for leptospirosis, all might have been associated with the 52% rate of cattle seroreactive to leptospirosis in the settlement.

Logistic regression analysis indicated that the variables presence of wetlands, rodents, dogs, cats and horses, along with occurrence of abortion, were associated with the risk of occurrence of antibodies to *Leptospira* spp.

Results reported by Zacarias et al. (2008) showed that leptospirosis in cattle can be transmitted by rodents infected with the serovar Copenhagen, and that cattle may pose a risk of transmitting the infection to humans. In the Brazilian state of

Maranhão, Silva et al. (2012) identified through multivariate logistic regression that the variables presence of horses and presence of capybaras were associated with the risk of occurrence of leptospirosis cases. Wild animals such as deer have also been associated with cases of bovine leptospirosis (Oliveira et al., 2010).

Leptospirosis prophylaxis includes the adoption of important procedures, such as control of synanthropic rodents, which are the main disseminators of the serovars Copenhagen and Icterohaemorrhagiae; elimination of excess stagnant water in the environment; isolation and treatment of animals with clinical history; detection and treatment of asymptomatic carriers; and systematic immunization of animals (Guimarães et al., 1983). Anti-rat measures should be carried out, such as adequate disposal of solid waste, proper storage and protection of food for human and animal use, proper disposal of trash, and rational use of long-acting rodenticides to eliminate synanthropic rodents (BRASIL, 2010).

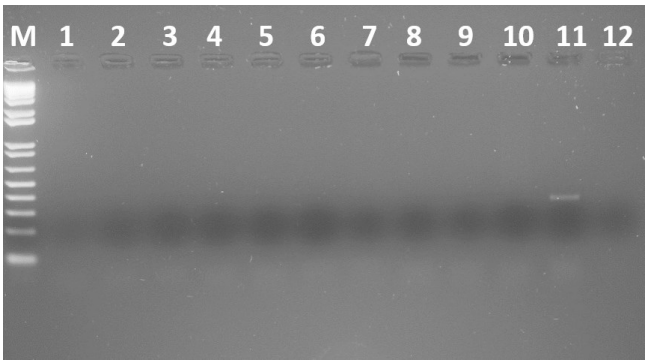

Figure 2. DNA extraction from urine samples from animals and from a pure culture of *Leptospira interrogans* serovar Hardjo, in EMJH medium. Molecular weight standard in ascending scale of 100bp (M); Urine samples (1 to 10); Pure culture sample of *Leptospira interrogans* serovar Hardjo in EMJH medium (11); and PCR reaction mixture without DNA, containing 10µL of ultrapure water (12).

Also, as an action to control infection by serovars of *Leptospira* spp. in cattle, antibiotic therapy was applied to all seroreactive animals in order to eliminate possible renal carriers and minimize the maintenance of leptospires in the herds. Vaccination was also used in treated and healthy animals, mainly in heifers before the reproductive phase, as recommended by Genovez et al. (2002).

In addition to its importance in animal health, leptospirosis can pose a risk to human health and family production systems, since it allows contact between humans and the secretions of infected animal.

4 | Conclusion

The study found that the presence of wetlands with access to cattle, presence of rodents, dogs and horses and occurrence of abortions were associated with the detection of antibodies against *Leptospira* spp. in the area of the settlement. We also carried out an awareness campaign among the farm families, proposing improvements in the sanitary conditions of the herds and rural properties in order to promote the health of the animals and families.

5 | Conflict of Interest

The authors declare that there is no conflict of interest.

6 | Ethics Committee

The study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine of Araçatuba (Process 004153/09).

7 | Referências

Bharti A.R. et al. Leptospirosis: a zoonotic disease of global importance. **Lancet Infectious Diseases**, 3(12): 757-771, 2003.

BRASIL. Ministério da Saúde. **Doenças Infecciosas e Parasitárias: Guia de Bolso**. 8ª ed. Brasília: Secretaria de Vigilância em Saúde, 2010. 444p.

Buainain, A.M.; Souza Filho, HM. **Procera: Impactos produtivos e capacidade de pagamento, Relatório Preparado para o MEPF**. Convênio FAO/INCRA, Brasília, 1998.

Castro, V. et al. Soroprevalência da leptospirose em fêmeas bovinas em idade reprodutiva no Estado de São Paulo, Brasil. **Arquivos do Instituto Biológico**, 75(1): 3-11, 2008.

Clazer, M. et al. Toxoplasmosis, leptospirosis, and brucellosis seroepidemiology in veterinary medical students and their relation with unique health. **Semina: Ciências Agrárias**, 38(3): 1347-1360, 2017.

Cosate, M.R. et al. Molecular typing of *Leptospira* interrogans serovar Hardjo isolates from leptospirosis outbreaks in Brazilian livestock. **BMC Veterinary Research**, 13(177): 1-12, 2017.

Dewes, C. et al. Isolamento de *Leptospira* em feto equino abortado. **Brazilian Journal of Development**, 6(8): 62347-62354, 2020.

Di Azevedo, M.I.N. et al. Characterization of *Leptospira*l DNA in the follicular fluid of non-pregnant cows. **The Veterinary Record**, 188(9): e143, 2021.

Diz, F.A.; Conceição, G.M.S. Leptospirose humana no município de São Paulo, SP, Brasil: distribuição e tendência segundo fatores sociodemográficos, 2007–2016. **Revista Brasileira de Epidemiologia**, 24: 1-14, 2021.

Faine, S. **Guidelines for the control of leptospirosis**, World Health Organization, Geneva, 1982. 171 p.

Favero, M. et al. Leptospirose bovina - variantes sorológicas predominantes em colheitas efetuadas no período de 1984 a 1994 em rebanhos de 21 Estados do Brasil. **Arquivos do Instituto Biológico**, 68(2): 29-35, 2001.

Favero, J.F. et al. Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. **Microbial Pathogenesis**, 107: 149-154, 2017.

Ferreira, L.D.S. et al. Review of the main techniques of diagnostic of leptospirosis. **Brazilian Journal of Health Review**, 4(4): 15230-15243, 2021.

Fornazari, F. et al. *Leptospira* reservoirs among wildlife in Brazil: Beyond rodents. **Acta Tropica**, 178: 205-212, 2018.

Garba, B. et al. Retrospective study of leptospirosis in Malaysia. **EcoHealth**, 14(2): 389-398, 2017.

Genovez, M.E. et al. Serological profile of a nelore herd presenting endemic leptospirosis and submitted to vaccination. **Arquivos do Instituto Biológico**, 71(4): 411-416, 2004.

Guimarães, M.C.; Côrtes, J.A.; Vasconcellos, S.A. Epidemiologia e controle de leptospirose em bovinos. Papel do portador e seu controle terapêutico. **Comunicação Científica da Faculdade de Medicina Veterinária e Zootecnia, USP**, 6/7: 21-34, 1982/1983.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Sistema do Instituto Brasileiro de Geografia e Estatística de Recuperação Automática – SIDRA**. IBGE, 2024. Available at: <http://www.sidra.ibge.gov.br/bda/pecua/default.asp>. Accessed on: 16 jun. 2024.

Jain, L. et al. Seroprevalence of brucellosis in bovines of Chhattisgarh, India. **Indian Journal of Animal Research**, 53(2): 255-259, 2019.

Juliano, R.S. et al. Prevalência e aspectos epidemiológicos da leptospirose bovina em rebanho leiteiro da microrregião de Goiânia- GO. **Ciência Rural**, 30(5): 857-862, 2000.

Lage, A.P. et al. Serology for *Leptospira* sp. in cattle of the State of Paraíba, Brazil. **Arquivos do Instituto Biológico**, 74(3): 185-190, 2007.

Lucchesi, P.M. et al. Recommendations for the detection of *Leptospira* in urine by PCR. **Revista da Sociedade Brasileira de Medicina Tropical**, 37: 131-134, 2004.

Luna, H.S. et al. Diagnóstico das condições do manejo sanitário e da saúde de bovinos criados no assentamento Vinte de Março localizado no município de Três Lagoas-MS. **Revista Saúde e Meio Ambiente**, 10(1): 32-42, 2020.

Maiolino, S.R. et al. Sperm viability, serological, molecular, and modified seminal plasma agglutination tests in the diagnosis of *Leptospira* in the semen and serum of bovine bulls. **Brazilian Journal of Microbiology**, 52(4):2431-2438, 2021.

Manzini, S. et al. First isolation of *Leptospira* kirschneri serovar canicola and *Leptospira* interrogans serovar pyrogenes in urine samples from slaughtered cattle in midwest region of São Paulo state, Brazil. **Archives of Veterinary Science**, 26(1): 51-62, 2021.

Martins, M.H.M.; Spink, M.J.P. Human leptospirosis as a doubly neglected disease in Brazil. **Ciência & Saúde Coletiva**, 25(3): 919-928, 2020.

Mérien, F. et al. Polymerase chain reaction for detection of *Leptospira* spp. in clinical samples. **Journal of Clinical Microbiology**, 30(9): 2219-2224, 1992.

Miller, D.A. et al. Porcine leptospirosis in Iowa. **Journal of Veterinary Diagnostic Investigation**, 2(3): 171-175, 1990.

Oca, O.Z.M.; Sanchez-Melorada, H.M.; Guerrero, A.V.M. La rata en la epizootiologia de la leptospirosis en granjas porcinas. **Técnica Pecuária en México**, 52: 29-44, 1986.

Oliveira, F.C.S. et al. Fatores de risco para a leptospirose em fêmeas bovinas em idade reprodutiva no Estado da Bahia, Nordeste do Brasil.

Pesquisa Veterinária Brasileira, 30(5): 398-402, 2010.

Oliveira, S.V.; Arsky, M.L.N.S.; Caldas, E.P. Reservatórios animais da leptospirose: Uma revisão bibliográfica. **Saúde** (Santa Maria), 39(1): 9¬20, 2013.

Pietrafesa, J.P.; Alves, A.I.; Pietrafesa, P.A. The Social Division of Labor in Rural Spaces in Brazil: Memory and History of the Expansion of Rural Social Movements and Disputes Over Hegemony. **Fronteiras: Journal of Social, Technological and Environmental Science**, 7(2): 202-224, 2018.

Pinheiro, F.P. et al. Infectious diseases along Brazil's Trans-Amazon highway: surveillance and research. **Bulletin of the Pan American Health Organization**, 8: 111-122, 1974.

Ribeiro, S.C.A.; et al. Leptospirose no rebanho bovino da sub-região de Nhecolândia, Pantanal Matogrossense, Brasil. **Veterinária Notícias**, 5(1): 51-55, 1999.

Rahman, T. et al. Zoonotic Diseases: Etiology, Impact, and Control. **Microorganisms**, 8: 1405, 2020.

Richtzenhain, L.J. et al. A multiplex PCR for the detection of *Brucella* spp. and *Leptospira* spp. DNA from aborted bovine fetuses. **Veterinary Microbiolgy**, 87(2): 139-147, 2002.

Santa Rosa, C.A. Diagnóstico laboratorial das leptospiroses. **Revista de Microbiologia**, 1(2): 97-109, 1970.

Santa Rosa, C.A. et al. Leptospirosis in wildlife in Brazil: isolation of serovars Canicola, Pyrogenes and Grippotyphosa. **International Journal of Zoonosis**, 7: 40-43, 1980.

Sant'ana, A.L. et al. Estratégias de Produção e Comercialização dos Assentados da Região de Andradina, Estado de São Paulo. **Informações Econômicas**, 37(5): 29-41, 2007.

Silva, F.J. et al. Prevalência e fatores de risco de leptospirose bovina no Estado do Maranhão. **Pesquisa Veterinária Brasileira**, 32(4): 303-312, 2012.

Thompson, J.A. et al. Spatial hierarchical variances and age covariances for seroprevalence to *Leptospira* interrogans serovar hardjo, BoHV-1 and BVDV for cattle in the State of Paraíba, Brazil. **Preventive Veterinary Medicine**, 76: 290-301, 2006.

Vasconcellos, S.A. et al. Leptospirose bovina. Níveis de ocorrência e sorotipos predominantes em rebanhos dos Estados de Minas Gerais, São Paulo, Rio de Janeiro, Paraná, Rio Grande do Sul e Mato Grosso do Sul, período de janeiro a abril de 1996. **Arquivos do Instituto Biológico**, 64(2): 7-15, 1997.

Veloso, I.F. et al. A comparison of three DNA extractive procedures with *Leptospira* for polymerase chain reaction analysis. **Memórias do Instituto Oswaldo Cruz**, 95(3): 339-43, 2000.

Zacarias, F.G.S. et al. Isolation of *Leptospira* serovars Canicola and Copenhageni from cattle urine in the state of Paraná, Brazil. **Brazilian Journal of Microbiology**, 39(4): 484-488, 2008.