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# Prevalence and risk factors associated with anti-*Leptospira* spp agglutinins in cattle from dairy farmers in Ji-Paraná, RO, Brazil

[*Prevalência e fatores associados ao risco da presença de aglutininas anti-Leptospira spp em bovinos de agricultores familiares da bacia leiteira do município de Ji-Paraná – RO, Brasil*]

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## ABSTRACT

Leptospirosis affects several animal species, including man. This study aimed to evaluate the prevalence of *Leptospira* spp. and to identify factors associated with the risk of *Leptospira* spp. in dairy cattle in the municipality of Ji-Paraná, RO, Brazil, sampled by rural sector, from September 2012 to November 2013. Blood samples from 627 dairy cows were randomly collected from 63 farms belonging to six rural sectors. Leptospirosis was diagnosed by the microscopic serum agglutination technique. Of the 627 animals tested, 255 had anti-*Leptospira* antibodies (40.48%, 95% CI: 36.64-44.31) and 57 of the 63 studied dairy farms (90.5%, 95% CI: 83.23-97.72) had at least one reactive animal. The results indicate that serovar Hardjo had the highest (12.38%. 95% CI: 10.03-15.18) followed by serovars Shermani, Wolffi, Hebdomadis and Canicola occurrence in dairy cows. Additionally, infection was also associated with abortion occurrences in cows of 36 farms (57.14%) and the presence of dogs roaming free with access to pasture, water and cattle in 47 farms (74.60%). Therefore, free-roaming animals are considered a predisposing factor, highlighting the need for adopting prophylactic measures while raising the awareness from rural producers about the importance and the economic losses that leptospirosis may cause.

Keywords: leptospirosis, dairy cattle, hardjo, epidemiology

## RESUMO

*A leptospirose acomete diversas espécies animais, inclusive o homem. Este estudo teve como objetivo avaliar a prevalência de anticorpos contra Leptospira spp., bem como identificar fatores associados ao risco da infecção por Leptospira spp. em bovinos de leite do município de Ji-Paraná – RO, amostrados por setor rural, de setembro de 2012 a novembro de 2013. Amostras de sangue foram coletadas sistematicamente de 627 fêmeas leiteiras, oriundas de 63 propriedades pertencentes a seis setores rurais. O diagnóstico da leptospirose foi realizado por meio da soroaglutinação microscópica. Constatou-se que 255 animais possuíam anticorpos anti-Leptospira (40,48%, IC95%: 36,64-44,31). Das 63 propriedades estudadas, 57 (90,5%, IC95%: 83,23-97,72) apresentavam pelo menos um animal reagente. Observou-se maior ocorrência do sorovar Hardjo nas fêmeas bovinas (12,38%, IC95%: 10,03-15,18), seguido dos sorovares Shermani, Wolffi, Hebdomadis e Canicola. Observaram-se, como fatores associados à infecção por sorovar, a ocorrência de aborto em fêmeas de 36 propriedades estudadas (57,14%), bem como a existência de cães criados livres em 47 propriedades (74,60%) e com acesso ao pasto, à água e aos bovinos, o que reforça a necessidade de adoção de medidas profiláticas e a conscientização dos produtores rurais sobre a importância e os prejuízos que podem ser causados pela leptospirose.*

*Palavras-chave: leptospirose, bovinocultura de leite, Hardjo, epidemiologia*

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## INTRODUCTION

Infectious diseases that may cause reproductive problems such as abortions, stillbirths, infertility, mastitis, and even death resulting in serious economic losses in dairy herds and are very important from the economic viewpoint (Pasqualotto *et al*., 2015; Mineiro *et al*., 2014). Leptospirosis is a zoonosis caused by bacteria of the genus *Leptospira* (Grooms, 2006) and can affect domestic and wild animals, and humans as well (Bharti *et al*., 2003; Aguiar *et al*., 2007, 2008, 2010; Santos *et al*., 2016). This disease is widely and easily spread especially by asymptomatic animals eliminating the microorganism in the urine for varying periods, keeping the disease endemic in the properties (Genovez, 2016).

The carrier status is important for the zoonotic potential and represents a greater concern, especially for workers in direct contact with animals and their secretions and excretions, as well as farmers, slaughter workers, and veterinarians as well (Faine *et al*., 1999; Genovez, 2016). Identifying the *Leptospira* serological variant is very important because acquired immunity is serovar-specific, so immunization only protects against homologous or antigenically similar serovars (Levett *et al*., 2001). The best control is vaccination with serovars prevalent in the region, otherwise, immunization is not effective (Melo *et al*., 2010).

In cattle, leptospirosis is considered one of the most important infectious diseases (Grooms, 2006; Miashiro *et al*., 2018) because it affects reproduction, causes abortions, embryonic death, stillbirths, infertility and even death of animals having, therefore, a great negative economic impact (Adler and Moctezuma, 2010). This results in direct economic losses related to a drop in milk production and a reduction in the growth rate, as well as indirect costs arising from expenditure on medicines and veterinary assistance (Faine *et al*., 1999; Fávero *et al*., 2001).

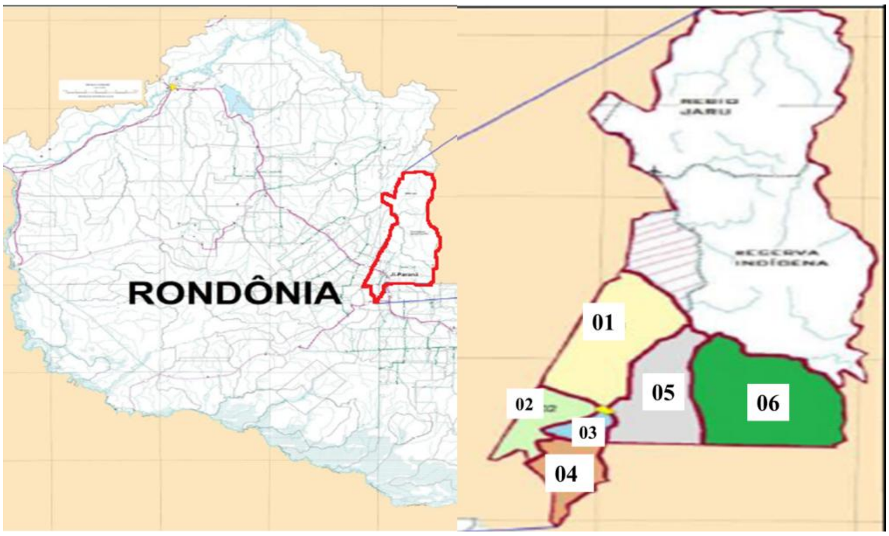
There are several studies in the literature assessing the occurrence and prevalence of leptospirosis in various South American countries (Ochoa *et al*., 2000; Alfaro *et al*., 2004; Léon *et al*., 2009; Van Balen *et al*., 2009; Arias *et al*., 2011; Suepaul *et al*., 2011; Gonzales and Rivera, 2015) that report results ranging from 2.6 to 80.5% among different countries. In Brazil, surveys reveal occurrence rates ranging from 18.9 to 65.5% in the different states of the country (Favero *et al*., 2001; Marques *et al*., 2010; Oliveira *et al*., 2011; Pimenta *et al*., 2014; Chiebao *et al*., 2015; Paim *et al*., 2016; Miashiro *et al*., 2018).

Considering that these data are still incipient in some areas of the country, this study aimed to identify the prevalence and factors associated with the risk of leptospirosis infection in cattle from dairy farms sampled per rural sector, from September 2012 to November 2013 in the city of Ji-Paraná, RO, Brazil.

## MATERIAL AND METHODS

This research used the same samples and followed the same methodology for sample size calculation of the study by Boas *et al*. (2015) collected from September 2012 to November 2013 in the city of Ji-Paraná, RO, Brazil.

Ji-Paraná currently has an estimated population of 127,907 people (IBGE, 2018) in an area of 6,896,649 km² located west of the Brazilian Amazon (10°52’42” S and 61°56’41” W). The rural area of Ji-Paraná is divided into six rural milk production sectors (Fig. 1), according to data from the Technical Assistance and Rural Extension Company of the State of Rondônia (Empresa Estadual de Assistência Técnica e Extensão Rural do Estado de Rondônia/Emater, RO).




Source: Adapted (Boas *et al*., 2015).

Figure 1. Location of Ji-Paraná, RO, and the respective six rural milk production sectors according to data from EMATER, RO.

The sample size was calculated based on the study by Boas *et al*. (2015) and the number of cows older than 24 months with dairy aptitude from rural properties in Ji-Paraná, RO, using the following statistical equation:

$$n_0 = \frac{Np(1-p)}{(N-1)\left(\frac{d}{\approx\alpha/2}\right)+p(1-p)} \text{deff}$$

Where:
N = 34527 is the population size (bovine females ≥ 24 months).
P = 0.5 is the estimated prevalence (since there were no previous studies and the maximum number of occurrences with normal distribution would be about 50%).
d = 0.05 is the maximum error of the estimate.
Za/2 = 1.96 is a pre-defined value of the normal distribution.
*deff* = 1.5 is the design effect.

The sample size was defined as 570 animals and increased by 10% to 627 animals to account for probable sample losses. Subsequently, we fixed the number of 10 dairy cows to be sampled from each family farm (Primary Sampling Unit - UPA) so that the number of sampled properties was established as described below (Table 1).

$$N\ of\ farms = \frac{N}{10} = \frac{627}{10} = 62.7 = 63$$

Table 1. Six rural milk production sectors, number of farms, number of dairy cows, and sampled farms per sector in Ji-Paraná, RO, between 2012 and 2013

| Sector | Family farms | Dairy Cows | Sampled farms |
|---|---|---|---|
| 1 | 122 | 5.246 | 9 |
| 2 | 124 | 5.952 | 9 |
| 3 | 54 | 2.268 | 4 |
| 4 | 176 | 8.096 | 13 |
| 5 | 143 | 4.433 | 11 |
| 6 | 237 | 8.532 | 17 |
| Total | 856 | 34.527 | 63 |

Both, the farm from each rural sector and the dairy cows from each family farm, were selected by systematic drawing. The dairy farms were identified through a Satellite Georeferencing System (GPS).

The microscopic serum agglutination test (MSA) was performed using the live antigen collection, which includes 24 serological variants (sv) of leptospires, of which 22 are pathogenic and two are saprophytes (Santa Rosa, 1970). An animal was considered positive when reactive to at least one *Leptospira* spp. serovar regardless of the detected serovar and cases of co-agglutination.

We also adapted an epidemiological questionnaire regarding several factors related to different diseases that were filled out by personnel of the studied farms. However, only data related to leptospirosis were used in this work such as: type of breeding system, technical advice, abortion on the farm, disposal of dead animals, presence of dogs on the property, access of dogs to cattle, presence of other domestic animal species, rodent control, vaccination of animals against leptospirosis and topography of the property.

The descriptive statistical analysis was performed to determine frequency distribution and the confidence interval of occurrence rates, using the methodology recommended by Thrusfield (2010). The comparison between the occurrence rates in the family farms and sectors, considering the *Leptospira* spp result, yes or no, was performed using the Chi-square ($X^2$) test or Fisher exact test. To verify whether the variables were associated with the frequency of infection for each serovar, considering the factor versus the analyzed serovar, univariate analysis was performed to calculate the prevalence ratio relative risk and respective confidence interval followed by Chi-square ($X^2$) test or Fisher exact test, using the software Epi Info 7.

This study was registered and approved by the Ethics Committee on Animal Research (CEPA-UFMT), protocol number 23108.015662/12-5, on May 17, 2012.

## RESULTS AND DISCUSSION

Of the 630 cows sampled, 255 (40.48%; 95% CI: 36.71- 44.36) were serum-reactive. This value is lower than the 95.3% and 97% infection frequency/rates obtained in mixed cows in Monte Negro, RO, and in the state of Pará, respectively, by Aguiar *et al*. (2006) and Homem *et al*. (2001). However, our result is relatively close to the 66.2% and 65.5% infection rates determined for cattle in the state of Pará by Negrão *et al*. (1999) and Chiebao *et al*. (2015), respectively. It is noteworthy that the infection rates vary even within the same region due to several factors that can directly or indirectly influence the occurrence frequency of leptospirosis such as rainfall, topography that facilitates water accumulation, presence of rodents.

In the literature, several researchers also reported higher infection rates in several Brazilian states such as 61.0% (Favero *et al*., 2001), 89% (Viegas *et al*., 2001) and 77.9% (Oliveira *et al*., 2011) in Bahia. Furthermore, infection rates of 62.5% (Favero *et al*., 2001) and 61.1% (Pimenta *et al*., 2014) were observed in Mato Grosso and Paraíba, respectively. Marques *et al*. (2010) and Paim *et al*. (2016) found 62.2% and 18.9%, respectively, in the state of Goiás. Results like this research were found by Favero *et al*. (2001), 41.3% in Rio de Janeiro and Minas Gerais; and 41.2% in Tocantins, evidencing the wide occurrence with relatively high infection rates (close to or above 50%) in several Brazilian states.

Of the 63 farms studied, 6 (9.52%; 95% CI: 2.27-16.77) had no animal reactive to leptospirosis through MSA and were considered negative for the disease while 57 farms (90.47%; 95% CI: 83.23-97.72) were positive and had one or more animals reactive to *Leptospira* spp. The infection rates ranged from 10 to 90% within each family farm. Considering only the positive properties, i.e., those with animals reactive to the test, 47% had 50% or more of the sampled animals reactive to the bacteria. The percentage of farms with reactive animals and the percentage of reactive animals in the farms are very high and may represent a risk to animal health since leptospirosis can cause several reproductive disorders and consequent production losses.

The results show that out of the six sectors evaluated, 6 (100%; 95% CI: 60.96 -100) were positive for having at least one farm with at least one animal reactive to leptospirosis through MSA. The infection rates per sector are shown in Table 2.

Table 2. Number of properties per sector with animals reactive to leptospirosis serovars determined by the microscopic serum agglutination test, Ji-Paraná-RO, 2019.

| Sector | Farms with reactive animals | Prevalence* (%) | 95% Confidence Interval | Reactive animals | Animal prevalence (%) |
|---|---|---|---|---|---|
| 1 | 7 (9) | 77.78[b] | 45.26-93.68 | 20 (90) | 22,22[a] |
| 2 | 5 (9) | 55.56[c] | 26.66-81.12 | 12 (90) | 13,33[a] |
| 3 | 4 (4) | 100[a] | 51.01-100 | 24 (40) | 60,00[b,c] |
| 4 | 13 (13) | 100[a] | 77.19-100 | 71 (130) | 54,61[b] |
| 5 | 11 (11) | 100[a] | 74.11-100 | 45 (110) | 40,91[c] |
| 6 | 17 (17) | 100[a] | 81.57-100 | 83 (170) | 48,82[b,c] |
| Total | 57 (63) | 90,48 | -- | 255 (630) | 40,48 |

* Different letters in the column mean significant difference ($p<0.05$).

The percentage of sectors positive for leptospirosis with at least one property and at least one reactive animal ranged from 55.56 to 100%. The highest infection rates were determined for sectors 3, 4, 5 and 6, where all farms had seropositive animals. The frequency of *Leptospira* spp was significantly different ($p<0.05$) in the sectors.

Likewise, Favero *et al*. (2001) found 54.5% in the state of Rondônia; Homem *et al*. (2001) 61.2% in Pará; Aguiar *et al.* (2006) reported a 14.5% frequency in Monte Negro in the state of Rondônia and Miashiro *et al*. (2018), 79.80% in nine municipalities in Mato Grosso do Sul.

In this study, the percentages of serovars reactive to leptospirosis per serovar ranged from 0.16 to 12.38%, and the titers ranged from 1/100 to 1/1,600. The five serovars (Hardjo, Shermani, Wolffi, Hebdomadis and Canicola) with the highest occurrence were more frequent in sectors 4, 5, and 6. From the serovar collection used, the serovar Hardjo had the highest occurrence rate (12.38%; 95% CI: 10.03 -15.18) in dairy cows while the serovars Andamana, Batavie, Bratislava and Javanica were not detected in the evaluated cattle. Also, the serovar Wolffi had a high occurrence (11.43%; IC 95%: 9.17-14.5) in the animals evaluated, similar to 16.7% observed by Viegas *et al*. (2001) in Bahia; 12.3% in Monte Negro reported by Aguiar *et al*. (2006) in Rondônia; in addition to 12.8% in Mato Grosso do Sul by Miashiro *et al*. (2018). Other significant serovars found in this study were Shermani (11.59%; 95% CI: 9.32-14.32), Hebdomadis (10.85%; 95% CI: 8.65-13.52), and Canicola (8.25%; 95% CI: 6.35-10.66).

Several risk factors were associated with infection by *Leptospira* spp. (Table 3). Of the 63 farms surveyed, only two (3.17%) had their animals vaccinated against leptospirosis, indicating that the animals evaluated came into contact with *Leptospira* spp. at some point in their lives and that the results found through MSA are not a vaccine immune response (false positives).

Table 3. Factors associated with the risk of the presence of leptospiral serovars in the analyzed cattle, Ji-Paraná-RO, 2019

| Serovar | Frequency | Risk factor |
|---|---|---|
| *Leptospira* spp. | (p:0.0294; RR: 1.96 ICRR: 1.52-2.54) | Dogs present on the property/Sector |
| Shermani | (p:0.04; RR: 1.59 ICRR: 1.04-2.42) | Pigs present in the property |
| Hardjo | (p:0.0318; RR: 2.06 ICRR: 1.10-3.87) | Pigs present on the property |
| Hebdomadis | (p:0.0196; RR:1.35 ICRR:1.08-1.68) | Destination of dead animals |
| Hebdomadis | (p:0.0410; RR: 1.60 ICRR: 1.05-2.47) | Dogs present on the property |
| Canicola | (p:0.010; RR: 1.32 ICRR: 1.06-1.65) | Sheep present on the property |
| Canicola | (p:0.0087; RR: 0.43 ICRR 0.23-0.80) | Dogs present on the property |

The presence of dogs on the farm was a factor associated with the risk of infection by the serovar Canicola (p:0.0087; RR: 0.43 ICRR 0.23-0.80) (Table 3) as evidenced in this study. Considering that dogs are the main reservoirs of serovar Canicola, the epidemiological data are in line with the findings, since of the 63 properties surveyed, 47 of them (74.60%) had dogs, and in 44 of these 47 (93.61%) farms, the dogs were roaming free with access to pasture, water and cattle, thus facilitating the transmission. Corroborating Aguiar *et al*. (2007) that detected 10% of serovar Canicola in dog serum in the municipality of Monte Negro - RO. Likewise, Oliveira (2011) also reported that dogs present on the farm are a risk factor for the occurrence of bovine leptospirosis in a study conducted in the state of Bahia.

This disease is linked with the increasing occurrence of abortion and, consequently, with the disposal of aborted fetuses. Despite the high frequency and history of abortion on the farms, in this study, disposal was not identified as a risk factor, however, 53 properties (84.12%) did not dispose of the fetuses correctly/sanitarily since they were left on the pasture. Only seven properties (11.11%) burned or buried the fetuses while in three farms (4.76%) the fetuses were fed to the fish reared in tanks. Thus, the disposal of dead animals was a risk factor associated with the infection by the serovar Hebdomadis (p:0.0196; RR:1.35 ICRR:1.08-1.68) (Tab. 3).

The results show that the cattle breeding system adopted by rural producers was also related to the occurrence of leptospirosis in cattle. Of the 63 farms, 51 (80.95%) reared the animals extensively, 10 (15.87%) semi-extensively while 2 (3.17%) had a semi-intensive rearing system. Occasionally, these systems allow small ruminants to be reared with cattle, cohabiting pastures and providing contact between the two species that may favor intra-species transmission of infectious agents. Of the 63 farms, nine (14.28%) had sheep, corroborating our findings that the presence of sheep on the property was a risk factor associated with the infection by the serovar Canicola (p:0.010; RR: 1.32 ICRR: 1.06-1.65) (Tab. 3). Aguiar *et al*. (2010) detected a prevalence of 33.3% for leptospirosis in sheep serum and indicated that the serovar Canicola has already been described as responsible for serological reactions in sheep in the municipality of Monte Negro, RO, in Brazil.

The serovars Hardjo (12.38%) and Shermani (11.59%) were found more frequently in this study. The simultaneous presence of pigs on the farms was also considered a risk factor for infection by the serovars Hardjo (p:0.0318; RR: 2.06 ICRR: 1.10-3.87) and Shermani (p:0.04; RR: 1.59 ICRR: 1.04-2.42) (Tab. 3). Reinforcing this result, Azevedo *et al*. (2006) reported that serovars Hardjo (54.2%) and Shermani (16.6%) were found in serum from pigs in the state of São Paulo.

Considering that *Leptospira* can survive longer in flooded soils, the conditions observed in the pickets of the farms were another risk factor for infection. The pickets were placed in humid soils in four (6.34%) of the farms, flooded pastures in one (1.58%), lowland regions in seven (11.11%), and regions of undulating topography (pastures in lowland areas and high regions) in 23 (36.50%). It is important to highlight that Ji-Paraná is located in the eastern Brazilian Amazon, an area characterized by high annual rainfall rates that favor water accumulation in these pastures while allowing the maintenance of the agent *Leptospira* in the environment, as previously reported by Levett *et al*. (2001); Adler and Moctezuma (2010).

The serovar Icterohaemorrhagiae detected in this study indicates a risk to public health so that prophylactic measures must be adopted in the municipality and especially in inhabited farms. Another worrisome fact is that among the 63 farms, 18 (28.57%) did not implement any rodent control in the farm installations. The occurrence of leptospirosis in dairy cows in Ji-Paraná, RO, raises concern, given its impact on public health and because 15.7% of confirmed leptospirosis infections in humans between 2007 and 2016 had rural origin in Brazil (Brazil, 2018), in addition to the economic losses caused to the municipal and state dairy sector.

## CONCLUSIONS

The farms studied in the municipality of Ji-Paraná, RO, have a high infection rate of bovine leptospirosis. The serovars Hardjo, Shermani, Wolffi, Hebdomadis and Canicola were the most prevalent in this study. Some of these serovars are not found in available commercial vaccines, therefore, the importance of making specific vaccines available for serovars present in the regions is highlighted, so that the animal's immune response is, in fact, effective. Also, it is necessary to raise the awareness of farmers and people involved in the segments of the milk production chain to the importance of the disease, the economic losses due to the infection, and, consequently, the value of the prophylactic measures that must be adopted to control and eradicate this disease.

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