Epidemiological aspects in leptospirosis. Research of anti-*Leptospira* spp antibodies, isolation and biomolecular research in bovines, rodents and workers in rural properties from Botucatu, SP, Brazil

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Abstract

Leptospirosis is a worldwide infection, transmitted between man and animals that causes a decrease in the production of bovine flocks, and offer risks for public health, as an important zoonosis. The rodents are the main reservoirs of leptospires. It was studied 27 dairy farm properties located in or near from Botucatu-SP, Brazil. In these farms were collected blood and kidney samples from rodents, blood and urine samples from bovines and blood samples from the workers. The serology was performed with microscopic agglutination test (MAT). Samples of bovine urine and rodent kidneys were cultivated searching for leptospires isolation. The polymerase chain reaction (PCR) of the kidneys of the rodents was performed. In MAT, 46/140 (32.85%) bovine and 8/34 (23.53%) human sera samples were positive, respectively. In human samples, the serovar Brastilava (37.51%) presented the highest occurrence, while in bovines, the serovars Hardjo and Castellonis were most frequent, with 26.08% each one. All of the rodents were negatives in serology. No leptospire was isolated, and kidney samples were negative in PCR. In bovines, the dam water and the bad hygiene quality of milking process were considered important risks of infection in the affected properties ($p<0.05$), where other reproductive problems, except abortion, can be related. In other side, to human beings the drainage system was the most important risk factor in the studied properties. Thus, it was verified the necessity of an improvement in zoosanitary handling of the properties, mainly of water supply.

Key words:
Leptospira.
Zoonosis.
Occupational disease.
Bovines.
Rodents.

Introduction

Leptospirosis is a widespread anthropozoonosis, with a great importance to bovine herds, causing reductions in fertility and production of milk, and an increase of mortality in infected herds. The genus *Leptospira* include two species, *Leptospira interrogans*, pathogenic for animals and humans, and *Leptospira biflexa*, saprophyte. Its dissemination is characterized by the existence of asymptomatic carriers.[1]

Rodents have an important role as reservoirs. Brown rat, *Rattus norvegicus*, is the most important transmitter of this zoonosis to man.[1]

The human-to-human transmission is rare, and these can acquire the infection for direct contact with leptospire from urine, blood and organs of infected animals, or indirectly, from contaminated water and/or humid soil or vegetation. The most frequent form of human transmission is the exposition to urine of the infected animals, directly or indirectly. Water and food have an important role in the transmission of leptospirosis. Some professional groups are more exposed to the risk of acquiring the

infection by frequent contact with infected animals.[2]

In south region of scrub of Mato Grosso state, Madruga, Aycardi and Putt[3] found 505 (75.30%) bovine sera samples reactives in 670 tested samples. The prevalent serovars were Hardjo, Sejroe, Wolffi, Australis and Gryppotyphosa. On the other side, Vasconcellos et al.[4], in a study realized in 56 properties of the states of South, South East and Center-West regions from Brazil, with sera of 2448 animals obtained 75.00% of positivity to serovar Hardjo, 8.92% to Wolffi and 3.57% to Pomona.

Langoni et al.[5], studying 2761 samples from São Paulo and Paraná states, obtained 45.56% of positivity, where serovar Hardjo was prevalent. On the other side, Rodrigues, Muller and Freitas[6] found 71.43% among 14 dairy properties studied in Paraná state, with 166/1253 (13.25%) animals serologically reactive, being Icterohaemorrhagiae (28.91%), Pomona (21.08%) and Bataviae (16.87%) the most prevalent serovars.

Cabral and Langoni[7], researching the occurrence of leptospirosis in cows with or without mastitis, found 201/236 (85.17%) positive samples. The most frequent serovars were Hardjo (70.65%), Hebdomadis (56.22%), Wolffi (48.76%), Copenhageni (34.83%) and Icterohaemorrhagiae (32.84%). The authors isolated *Leptospira* spp in 12 (54.54%) urine samples of the positive animals serologically, as well as in 15 of 236 cultivated milk samples. This was the first description of isolation in Brazil of leptospire in milk.

Besides of its economic importance, leptospirosis assumes a distinction role in relation to public health, because many cases of human infection from bovines were reported, as well as in other professionals of risk activities, as the employers of slaughterhouse and cut sugar cane plantations[8]. It is an occupational disease with a higher frequency in some professionals groups, and it is associated to recreational activities too, performed in rural areas.[9]

Carvalho, Ávila and Girio[10] performed a serological inquiry in meat handlers, in Ribeirão Preto region and found positivity in 60.00% of slaughterers, 26.90% of assistants of production, 20.00% of reception area for bones and blood, 15.00% of deboning area and 14.30% of area to guts. In spite of leptospirosis being considered as an occupational disease, cases of its occurrence in workers, milkers, and veterinarians are rare. Bolin and Koellner[11] registered a case in a veterinarian that contaminated himself during necropsy of a bovine infected by serovar Hardjo. She had clinical disease and the serology presented titer 6400. During this period, breast-fed a child that developed disease 21 days after. This fact confirm the professional risk of the disease and the transmission of leptospire by milk.

Cordeiro[12] isolated leptospire in 46/116 (39.6%) mice (*Mus musculus*), in Rio de Janeiro. Caldas, Fehringer and Sampaio[13], in Salvador, evaluated 30 sera of *Rattus norvegicus* and five of *Didelphis marsupialis*. They found 20.00% positivity in both species, being Grippotyphosa and Icterohaemorrhagiae the most frequent serovars. Riedmann, Cabezas and Zamora[14] found 26/116 (22.40%) rodents serologically reactive, in rural areas of Chile, being Hardjo and Pomona the most frequent serovars. Zamora et al.[15] found 133/368 (36.10%) rodents, captured in Valdívia, Chile, serologically reactive to indirect fluorescence antibody test (IFAT). *Akodon longipilis* and *Akodon olivaceus* were the most important captured species of rodents, followed by *Oryzomia longicaudatus*, *Rattus rattus* e *Mus musculus*.

Polymerase chain reaction (PCR) has been used to detection of leptospires and other microorganisms in urine samples.[16,17] Kee et al.[18], studying the prevalence of leptospires in wild rodents found 22/222 (9.91%) *Apodemus agrarius* positives to microbiological culture and six animals were positive to PCR. Fukunaga et al.[19] determined the sequence of gene 16S rRNA of *Leptospira interrogans* serovar Canicola, Moulton strain and, from this sequence,

Mérien et al.[17] synthesized the primers LEP1 e LEP2, to detect the genetic material of *Leptospira* spp in samples since 10 bacterias/ml.

The aim of this study was to evaluate the taxes of infection in human and animals from different dairy properties, and to verify the relation among the infection in each studied property. We aimed too the detection of *Leptospira* spp in bovine urine for microscopic culture, and in renal tissue of captured rodents for microbiological culture and PCR.

Materials And Methods

Twenty-seven dairy farm properties from Botucatu-SP, and surrounding area were chosen randomly for this study, in 2005. Ten traps, jail type, were placed at nocturnal period, for each property, capturing a total of 50 rodents. The blood samples of the rodents were obtained for the puncture of the orbital sinus, and after the rodents were sacrificed and collected kidneys to the attempt of isolation of leptospires, and to PCR, maintaining at -20ºC until the moment of the test.

The samples of bovine blood were collected by jugular puncture of 10% of the herd, from each property, being 10% of each category – heifers, cows in lactation, in dry period and male, in a total of 140 samples (5.18/property), being representative of these herds. Thirty-five samples preceded from farm properties that no one rodent was captured. Furosemide, 0.5mg/kg [20], intramuscular via, was used to stimulate the spontaneous urination, to collect the urine samples of bovines, adding these in sterile flasks with capacity of 20mL, directed immediately to the laboratory. Blood samples of 34 workers, which maintained contact with the animals, 1.70/property, were obtained for research anti-*Leptospira* spp antibodies. These blood samples were collected from farm properties, in which rodents were captured. Table 1 shows the localization of the farm properties and the distribution of the number of bovine, rodent and human samples.

Serology was realized by Microscopic Agglutination Test (MAT) according to the Ministry of Health.[1] Sera samples were diluted at 1:50 in phosphate buffered solution (PBS) 0,01M pH7.2 and tested with 24 serovars of *Leptospira* spp: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Brasiliensis, Canicola, Withcombi, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi e Andamana, maintained in EMJH liquid medium (DIFCO®), enriched with Bovine Albumin Fraction V. Positive results were considered to the sera samples that agglutinated 50% or more leptospires, realizing dilutions in ratio 2, to get the final titer of antibodies.

EMJH hemi-solid medium with bovine albumin fraction V was used to isolation.[1] Kidney samples were macerated in a porcelain vessel and diluted since 10^{-1} a 10^{-3} in PBS 0.01mL pH7.2, with 1% bovine albumine and 25mg/mL neomicine and furazolidone, in tubes with spiral cap. In 5mL of medium was inoculated 0.5mL of each dilution. Urine samples were diluted too, and cultivated as described above, and incubated for two months at 30ºC, being verified weekly a possible appearance of an opalescence ring, "Dinger's ring", as well as the presence of spirochete in culture with signs of bacterial growth, examining 25µL of culture medium of each tube in dark field microscope, with 40x objective.

The extraction of DNA was realized based in the protocol described for Janssen[21]. Each microtube of 0.2mL received 5mL PCR buffer (50mmol KCl, 10mmol Tris-HCl), 1.5mL $MgCl_2$ (1.5mmol), 8.0mL deoxynucleotides solution (1.25mmol), 1.5U *Taq* DNA polymerase, 10pmol each primer, 10mL each sample and 15.2mL ultrapure water. The primers described for Mérien et al.[17] were: LEP1: 5'GGCGGCGCGTCTTAAACATG3' and LEP2: 5'TTCCCCCCATTGAGCAAGATT3'. PCR starts with an initial extension at 94ºC

for 3 minutes, followed by 29 cycles with denaturation at 94°C for 1 minute, annealing at 63°C for 1.5 minutes and extension at 72°C for 2 minutes, finishing with an final extension at 72°C for 10 minutes. After, the amplification was evaluated in electrophoresis with 2% agarose gel diluted in 0.5x Tris-borate-EDTA buffer (TBE), being stained in ethidium bromide solution for 30 minutes, with visualization of bands in ultraviolet transluminator, with filter of 300nm.

A questionnaire of epidemiological investigation was applied to evaluate the conditions of each property. The conditions of the local were considered adequate when the animals were raised isolate, with water *ad libitum* and specific local to milk. The drainage was considered sufficient when the formation of pools or accumulative water and dejects, as feces and urine, wasn't observed. The conditions of food storage were considered good in cases of clean, aired locals, without humidity and possible shelter to rodents. The hygiene in milking process was good when there was a specific local to this process, occurring the washing of the teats before and the material used was apparently clean. The equipments used in milking process were gloves, masks and boots. The presence of feces in the local was observed to determine if the infestation for rodents was recent or old, considering old to dried up feces, and recent to humid and brilliant feces.

The taxes of infection and the presence of *Leptospira* spp were evaluated for descriptive statistic. The association among the results of MAT, in bovines and humans, and the epidemiological variables was verified for Chi-square or Fischer's exact test, with a=0.05 [22], using EPINFO 6.04c program.

Results

Fifty rodents were captured in 20 dairy farm properties of 27 researched properties (2.5/property), where 45 were *Rattus rattus* and only five *Mus musculus*. The culture of macerated renal material of the rodents, and the urine of bovines, as well as the research of anti-*Leptospira* spp antibodies in sera samples of captured rodents, were negative. At the same form, the samples of renal tissue of the rodents were also negative to PCR.

Forty-six bovine sera samples of 140 (32.85%) were reactives and the reactive serovars were Hardjo (26.08%), Castellonis (26.08%), Grippotyphosa (21.74%), Bratislava (19.56%), Wolffi (17.38%), Icterohaemorrhagiae (10.86%), Copenhageni (8.69%) e Canicola (4.34%). Eight human sera samples of 34 (23.53%) were reactives to serovars Bratislava (37.50%), Cynopteri (25.00%), Autumnalis, Butembo and Hebdomadis (12.50%) (Table 1).

The association among the obtained results with the questionnaire of epidemiological investigation, and the results of MAT, in bovines and humans, is presented in tables 2 and 3, showing the importance of the water source, hygiene of milking process and reproductive problems as important risk factors for bovines in these studied farm properties, as well as the drainage to human beings.

Discussion

The dairy farm properties presented the same characteristic with few crossbred animals and with low productivity. Equipments, tools, construction materials and ration were stored in deposits, providing ideal conditions to rodents. The breed of animals of other species was common, mainly swines and caprines, beyond the dogs with free access to this breeds and deposits of the properties.

According to the species of captured rodents, *Rattus rattus* is the most common specie in rural areas, due the habit of to shelter in trees, freeds and gaps of house, which is common in rural habitations.[1]

In bovines, as well as in other species, not ever the serological titers correspond to the elimination of leptospires in urine, and to the isolation in necessary a significative

Table 1 - Serology to leptospirosis by MAT in bovines, human beings and wild rodents from farms of Botucatu region, São Paulo, Brazil, according to farm identification, municipality, animal species, proportion of reactives and most frequent serovars. Sample collection in 2005. Botucatu, 2007

Property	Localization	Bovines			Humans			Rodents		
		N**	+***	Serovar	N**	+***	Serovar	N**	+***	Serovar
P1	Anhembi	05	01	3	00	00	----	00	00	----
P2	Anhembi	05	01	16A, 16B	02	00	----	02	00	----
P3	Anhembi	05	00	----	01	01	2A	04	00	----
P4	Anhembi	05	03	9, 16A, 16B	01	00	----	05	00	----
P5	Bofete	05	03	1B, 9	00	00	----	00	00	----
P6	Bofete	05	01	5, 9	01	00	----	03	00	----
P7	Bofete	05	02	16A, 16B	02	00	----	03	00	----
P8	Bofete	05	00	----	03	01	1B	02	00	----
P9	Bofete	05	00	----	00	00	----	00	00	----
P10	Botucatu	05	02	1B, 9	02	01	1B	03	00	----
P11	Botucatu	05	03	1B, 9	02	01	7	01	00	----
P12	Botucatu	05	00	----	01	00	----	02	00	----
P13	Botucatu	05	04	16A, 5	01	00	----	01	00	----
P14	Botucatu	05	01	11A, 3, 16B	01	00	----	01	00	----
P15	Botucatu	05	01	16A, 3	02	02	10, 2B	02	00	----
P16	Botucatu	05	03	16A, 16B, 11A	01	00	----	03	00	----
P17	Botucatu	05	04	3, 16B, 11B	00	00	----	00	00	----
P18	Botucatu	10	03	3, 9, 11B	06	00	----	03	00	----
P19	Botucatu	05	01	16A, 16B	01	00	----	04	00	----
P20	Pardinho	05	02	1B, 9	00	00	----	00	00	----
P21	Pardinho	05	00	----	00	00	----	00	00	----
P22	Pardinho	05	02	16A, 16B	00	00	----	00	00	----
P23	Pardinho	05	02	3, 11A, 14A	01	00	----	03	00	----
P24	Pardinho	05	04	16A	02	00	----	01	00	----
P25	Pardinho	05	01	9, 11B	01	00	----	03	00	----
P26	Rubião Júnior	05	00	----	01	00	----	01	00	----
P27	Rubião Júnior	05	02	16B, 11B, 1B	02	02	2A	03	00	----
Total	**27**	**140**	**69**	----	**34**	**08**	----	**50**	**00**	----

* P3, P5, P11, P12, P16, P21, P25 – properties where rodents were not captured
**tested samples, ***reactive samples
1B Bratislava, 2A Autumnalis, 2B Butembo, 3 Castellonis, 5 Canicola, 7 Cynopteri, 9 Gryppotiphosa, 10 Hebdomadis, 11A Copenhageni, 11B Icterohaemorrhagiae, 16A Hardjo, 16B Wolffi

bacterial load.[20,23]

Cabral e Langoni[7], trying to isolate leptospires from milk of cows with or without mastitis, observed that the growth of other bacterias can affect the development of leptospires. Its isolation from urine not even is possible; however the sucess increase when furosemide is used to stimulate the urination. However, in present study, the elimination of spirochetes by urine wasn't verified.

In kidneys, leptospires are protected against the action of antibodies, and thus stay for a long time in animals, and during for all the life in rodents.[22] In this way, the isolation or demonstration of leptospires is fundamental to evaluate the role of rodents in epidemiological chain of transmission of this disease. The negative result to the isolation of leptospires from kidneys, can be occurred for the same causes referred to urine samples, however kidneys were obtained with asepsis condition, in laminar flow chain, and this material could have a higher concentration of leptospires than urine.

Leptospire infection was detected in 77.78% studied herds. It shows a widespread of the agent in this region. These data are similar to those referred by Vasconcellos et al.[4] and Rodrigues, Muller and Freitas[6] that observed positivity in 75.00% and 71.43% studied herds, respectively. The perceptual of positive animals (32.85%) was lower than that found by Madruga, Aycardi and Putt[3] with 74.50%, Langoni et al.[5] with 45.50%, and Cabral e Langoni[7] with 85.17%. Only Cabral e Langoni[7] investigated in animals from the same region. On the other side, these data was higher than those found by Rodrigues, Muller and Freitas[6] with 13.25%, that studied sera samples from Paraná state.

In properties where serovar Hardjo was prevalent, probably the main responsible for the maintenance of the agent in the environment are bovines, once this serovar is adapted to this specie. When other serovars

present more frequency, as found by Rodrigues, Muller and Freitas[6], that obtained higher frequency of the serovars Icterohaemorrhagiae and Pomona, other species can be involved in the epidemiology, as respectively rodents and swine.

In present study, response to serovar Icterohaemorrhagiae wasn't found by human beings, concluding that rodents are not important in this case. At the same form that there were no human and bovine serologically reactive to the same serovar, at the same property, we believe in the hypothesis of the involvement of others animals in the dissemination of the disease. All rodents were negatives in MAT. Some authors obtained positivity higher than 50%; however there are few studies with *Rattus rattus* and *Mus musculus* in rural areas, mainly in farms. Smith et al.[24] studied mice captured in swine farms, and found prevalence higher than 43%.

The result of serology is few explained when evaluated alone, because leptospires cause a short time immune response, and the circulating antibodies weren't detectable by MAT in some months. Thus, the absence of serological titer doesn't mean that the animal isn't a reservoir, because these animals can eliminate leptospires in urine for all the life, without presenting detectable levels of circulating antibodies.[13]

All kidney samples of the rodents were examined by PCR, searching for genetic material of the agent, in spite of not have occurred the isolation of leptospires, because leptospires can be inactivated just after the samples collection. But all of samples were still negative to PCR, showing that in the studied area, *Rattus rattus* and *Mus musculus* have few or none importance in epidemiological chain of transmission of leptospirosis in dairy farm properties.

Rattus norvegicus is the main reservoir of leptospires, and *Rattus rattus* and *Mus musculus* respectively, with roof and domiciliary habits have a little contribution to the maintenance of leptospires in nature.[1] Other wild rodents, as *Akodon longipilis, Akodon olivaceus* and *Apodemus agrarius* can perform an important role in the transmission and maintenance of leptospires in nature.[15,18]

In the studied properties was common the breed of animals of other species at he same environment and, usually, dogs have access to storerooms and to places used as deposits, as well as water sources. In others, there was breed of swine too, and the water used for washing the piggery was conducted to the dam used as water source for animals, allowing interspecies transmission. Del Tedesco[25] agrees that the practices that allow the breed of more animals in a small area, contribute for the dissemination of the agent in herds. The conditions of the local didn't contribute for the dissemination of the agent in properties, even when different animal species were bred together with bovines, or had access to their herd. This shows that bovines were the main responsible for the maintenance of leptospires in these properties.

Leptospires remain in humid and inundate locals, but they aren't capable to survive for a long time where occurs intense fermentation, that cause a increase of temperature, and the reduction of pH, as in places with great amount of dejects, as feces and urine. This explains the tendency of the infection to be prevalent in locals with an insufficient drainage. In places with low amount of dejects, as dams, used as water source for animals, the stopped water was an important factor for the dissemination of the infection to the animals of the herd.

In all properties with good zoosanitary handling, the prevalence of anti-*Leptospira* spp antibodies was lower in bovines, reinforcing the importance of the aspects of animal sanity. It can be observed mainly during the hygiene of milking process and evaluating the water source. In this study, bad practices for hygiene of milking process were considered a factor risk with 41.4% animals from these properties reacted to MAT. These practices can disseminate the microorganism to the herd, contaminating the milk samples too. In the other side, 57.1% animals from properties with dams as water source reacted to MAT, which

Table 2 - Association among epidemiological variables and results of Microscopic Agglutination Test (MAT), to detection of anti-*Leptospira* spp antibodies in bovines from farms of Botucatu region, São Paulo, Brazil. Sample collection in 2005. Botucatu, 2007

Variable	Number of animals	% positives to MAT	χ^2	p	OR	Interpretation
Type of breed						
Extensive	125	32.8	-	0.80*	1.02	NS
Intensive	15	33.3				
Local conditions						
Inadequate	125	33.6	-	0.41	0.72	NS
Adequate	15	26.7				
Soil						
Land	130	33.0	-	0.57	0.87	NS
Rock/cement	10	30.0				
Drainage						
Sufficient	40	45.0	3.01	0.08	0.48	NS
Insufficient	100	28.0				
Water source						
Mine	105	24.8	11.05	0.0009	4.05	S
Dam	35	57.1				
Feeding						
Grass	75	37.3	1.06	0.30	0.64	NS
Ration	65	27.7				
Rodents						
Yes	125	33.6	-	0.41	0.72	NS
No	15	26.7				
Control of rodents						
Cat	65	35.4	0.17	0.68	0.81	NS
Raticide	75	30.7				
Milking process						
Manual	125	32.0	0.36	0.73	1.42	NS
Mechanical	15	40.0				
Hygiene of milking process						
Good	70	24.3	3.92	0.04	2.21	S
Bad	70	41.4				
Milking equipments						
No	130	33.1	-	0.57*	1.15	NS
Yes	10	30.0				
Occurrence of abortion						
No	40	37.5	0.29	0.58	0.75	NS
Yes	100	31.0				
Other reproductive problems**						
No	130	29.2	8.67	0.003	9.68	S
Yes	10	80.0				

χ^2=qui-square value (α=0.05); p=p value; OR=*odds ratio*; NS=no significative difference; S=significative difference; *when p values are not proceeded for qui-square value, they were calculated for Fischer's exact test; ** foetal deaths, mummified fetuses, estrus repetition, stillborn dead

can be explained by the free access of others animals to this source, contaminating the water (Table 2).

In this case, abortion, was not associated to the infection, but other reproductive problems as foetal deaths, stillborn deaths, estrus repetition, mummified fetuses, and others, were highly associated ($p<0.05$) to the research of anti-*Leptospira* spp antibodies in bovines, indicating that in this cases leptospirosis must be investigated in the herd, and some confirmatory tests should be performed. To humans only sufficient drainage was considered a risk factor, increasing the occurrence of the infection (Table 3).

The obtained results showed that leptospires was present in dairy herds of the studied region, and that the hygienic conditions of the milking process, as well as the origin of the water destined to cleanliness, and animal consumption are risk factors and probably routes of transmission of the microorganism. Thus, there is the necessity of an improvement in zoosanitary handling, with special care of water supply. The absence of any serological response to serovar Hardjo in human samples shows that the local strain has no preference for human beings.

Table 3 - Association among epidemiological variable and results of Microscopic Agglutination Test (MAT) to detection of anti-*Leptospira* spp antibodies in humans from farms of Botucatu region, São Paulo, Brazil. Sample collection in 2005. Botucatu, 2007

Variable	Number of humans	% positives to MAT	p	OR	Interpretation
Type of breed					
Extensive	26	23.1	0.62	1.11	NS
Intensive	8	25.0			
Local conditions					
Inadequate	31	19.4	0.13	8.33	NS
Adequate	3	66.7			
Soil					
Land	28	28.6	0.17	0.00	NS
Rock/cement	6	0.0			
Drainage					
Sufficient	10	50.0	0.03	0.14	S
Insufficient	24	12.5			
Water sources					
Mine	27	25.9	0.46	0.48	NS
Dam	7	14.3			
Control of rodents					
Cat	17	17.6	0.34	1.94	NS
Raticide	17	29.4			
Milking process					
Manual	25	28.0	0.29	0.32	NS
Mechanical	9	11.1			
Hygiene of milking process					
Good	12	8.3	0.13	5.13	NS
Bad	22	31.8			
Milking equipments					
No	28	28.6	0.17	-	NS
Yes	6	0.0			
Consumption of raw milk					
No	7	0.0	0.12	-	NS
Yes	27	29.6			
Occurrence of abortion					
No	12	33.3	0.27	0.44	NS
Yes	22	18.2			
Other reproductive problems*					
No	33	24.2	0.76	0.00	NS
Yes	1	0.0			

p=p value (calculated for Fischer's exact test; α=0.05); OR=*odds ratio*; NS= no significative difference; S= significative difference; * foetal deaths, mummified fetuses, estrus repetition, stillborn dead

Aspectos epidemiológicos nas leptospiroses: Pesquisa de anticorpos anti-*Leptospira* spp, isolamento e pesquisa biomolecular em bovinos, roedores e trabalhadores de propriedades rurais do Município de Botucatu, SP, Brasil

Resumo

A leptospirose é uma infecção amplamente difundida pelo mundo, transmitida entre o homem e os animais que causa uma queda na produção de rebanhos bovinos, e oferece riscos relacionados à saúde pública sendo uma importante zoonose. Foram estudadas 27 propriedades de pecuária bovina leiteira em Botucatu-SP e limítrofes, com colheitas de amostras de sangue e rim de roedores, sangue e urina de bovinos e sangue dos trabalhadores. A sorologia foi realizada pela soroaglutinação microscópica (SAM). Foi tentado o isolamento de leptospiras por cultura da urina dos bovinos e dos rins dos roedores. Foi realizada a reação em cadeia pela polimerase (PCR) dos

Palavras-chave:
Leptospira.
Zoonoses.
Doença ocupacional.
Bovinos.
Roedores.

rins dos roedores. Para a SAM, 46/140 (32,85%) amostras de soro bovino e 8/34 (23,53%) humana foram positivas, respectivamente. Para as amostras humanas, o sorovar Bratislava (37,51%) apresentou maior ocorrência, enquanto que para os bovinos, os sorovares mais freqüentes foram Hardjo e Castellonis, com 26,08% cada. Todos os roedores foram negativos à sorologia. Nenhuma leptospira foi isolada, e as amostras de rim foram negativas à PCR. Nos bovinos, a água de açudes e as ordenhadas com má qualidade de higiene foram consideradas importantes riscos de infecção nas propriedades acometidas ($p<0,05$), onde outros problemas reprodutivos, exceto aborto, podem estar relacionados. Por outro lado, para o homem o sistema de drenagem foi o mais importante fator de risco nas propriedades estudadas. Assim, enfatiza-se a necessidade de melhora no manejo zoosanitário das propriedades, principalmente com relação ao suprimento de água.

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