

Seroepidemiology of leptospirosis in pet dogs in the urban area of the municipality of Jataizinho, Paraná

Soroepidemiologia da leptospirose em cães domiciliados na área urbana do município de Jataizinho, Paraná

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Abstract

The study of the prevalence of anti-leptospiral antibodies in pet dogs in urban areas and the variables associated with the risk of infection can aid in the identification of the serovars present in the region and indicated the reservoirs involved in this important zoonosis. The aim of the present study was to determine the prevalence of anti-leptospiral antibodies in 653 dogs from 369 households distributed throughout the urban area of the municipality of Jataizinho, Paraná (PR), and to investigate the variables associated with the risk of leptospiral infection in these animals. Serum samples were subjected to microscopic agglutination test (MAT) against 23 serovars of pathogenic leptospire. Among the 132 (20.21%) reactive samples, 15 (11.36%) were reactive for more than one serovar, and the most likely serovar could not be determined. Among the 117 (88.73%) samples that reacted to a single serovar, the serovar Canicola was considered to be the most likely in 89 cases (76.07%). Variables associated with the risk of disease were investigated using an epidemiological questionnaire administered to each owner, and the data obtained were analyzed using the statistical software EpiInfo. The significant variables ($p \leq 0.05$) associated with seropositivity for leptospirosis in pet dogs in the urban area of the municipality of Jataizinho, PR, were the presence of rats in the household, adult or elderly dogs, free access of the dog to the street and contact with stray dogs. The results indicate a high prevalence of pet dogs in the urban area of Jataizinho, PR, with anti-leptospiral antibodies and suggest the need to educate the population of the municipality about the vaccination of dogs, as well as, the epidemiological role of the dog in urban leptospirosis.

Key words: Epidemiology, microscopic agglutination, test, *Leptospira* spp., serovar Canicola, diagnosis, risk variables

Resumo

O estudo da prevalência de cães domiciliados em áreas urbanas com anticorpos antileptospiras e de variáveis associadas ao risco de infecção, pode auxiliar na identificação dos sorovares presentes na região e sugerir os reservatórios envolvidos nesta importante zoonose. O objetivo deste trabalho foi determinar a prevalência de anticorpos antileptospiras em 653 cães de 369 domicílios distribuídos na área urbana do município de Jataizinho, PR, e estudar variáveis associadas ao risco de infecção por leptospiras nesses animais. As amostras de soro foram submetidas a prova de soroaglutinação microscópica (SAM)

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frente a 23 sorovares de leptospirosas patogênicas. Das 132 (20,21%) amostras de cães reagentes, 15 (11,36%) reagiram para mais de um sorovar, não sendo possível determinar o sorovar mais provável, e das 117 (88,73%) que reagiram para um sorovar somente, o sorovar Canicola foi considerado o mais provável em 89 (76,07%). Variáveis associadas ao risco da doença foram investigadas através de um questionário epidemiológico aplicado a cada proprietário e os dados obtidos foram analisados pelo programa estatístico EpiInfo. As variáveis significativas ($p \leq 0,05$) associadas à soropositividade para leptospirose nos cães domiciliados em área urbana do município de Jataizinho, PR, foram: visualizar ratos no ambiente familiar, animal adulto ou idoso, livre acesso do cão à rua e contato com cães errantes. Os resultados indicam uma alta prevalência de cães domiciliados em área urbana de Jataizinho, PR, com anticorpos antileptospirosas e sugerem a necessidade de conscientizar a população do município sobre a vacinação dos cães, assim como, o papel epidemiológico do cão na leptospirose urbana.

Palavras-chave: Epidemiologia, soroaaglutinação microscópica, *Leptospira* spp, sorovar Canicola, diagnóstico, variáveis de risco

Introduction

Leptospirosis is an important zoonosis caused by the spirochete *Leptospira interrogans* that affects all mammalian species. The disease has a worldwide distribution and can cause systemic infection with different clinical presentations, according the animal specie and the infecting serovar involved (FAINE et al., 1999).

Dogs are susceptible to all leptospiral serovars; however, when infected by serovar Canicola, they can act as asymptomatic carriers, eliminating the bacteria in the urine without any clinical presentation suggestive of leptospirosis (PRESCOTT, 2008).

In urban environments, dogs are the main reservoirs involved in the transmission of leptospirosis to humans and other dogs (BROD et al., 2005). Jansen et al. (2005) and Fraga (2008) associated the risk of human infection with the presence of dogs infected by leptospires inside the household or near it.

Several studies on pet and stray dogs have identified different variables associated with the risk of infection by leptospires, such as gender, age, presence of flooded areas near the household, contact with rodents, hunting habits and type of access to the street (RUBEL et al., 1997; MASCOLLI et al., 2002; QUERINO et al., 2003; LOPES et al., 2005; MAGALHÃES et al., 2007).

Rubel et al. (1997) consider that the transmission of leptospirosis in stray dogs can occur through direct contact with asymptomatic carriers or indirectly through contact with an environment contaminated by leptospires. Humid soils and stagnant water are important routes of infection for dogs that urinate, drink and walk over these microenvironments that favor the survival of leptospires for months (TRUEBA et al., 2004).

The immunization of dogs can prevent canine leptospirosis. Non-vaccinated populations can reach an incidence of disease of 75% of dogs (WARD, 2002).

Vaccines against leptospirosis are bacterins and produce a serovar-specific response, with no cross-protection between serotypes. Nonspecific vaccine titers can be detected by a microscopic agglutination test (MAT) up to three months after immunization (MILLER et al., 2011). Harkin et al. (2003) and Minke et al. (2009) showed that the vaccines prevent renal colonization, leptospiruria over long periods and death after extreme challenges in experimental infections.

The aim of the present study was to determine the prevalence and identify the risk variables associated with leptospirosis in pet dogs in the urban area of the municipality of Jataizinho, Paraná (PR).

Materials and Methods

Information on the city of Jataizinho, PR

The municipality of Jataizinho, PR, is located 79 km from Londrina, PR, with an area of 159.18 Km² and 11,875 inhabitants. According to the Brazilian Institute of Geography and Statistics (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE, 2010), 12.8% of permanent private households in the urban area have inadequate sanitation, and the monthly household income per capita in this area is R\$ 478.00.

The urban geographical area of the municipality was divided by the Epidemiological Surveillance, together with the Municipal Health Department, into five independent districts that correspond to the coverage area of the Basic Health Units (BHUs) in the municipality as a strategy for the implementation of health programs.

Households

In 2009, the urban area of Jataizinho-PR had 4,692 properties, of which 80% (3,754) were family households registered in the Family Health Program of the BHU, with 13,286 registered individuals and an average of 3.54 individuals per household.

Sample size

In urban areas, the human:dog ratio is 4:1, and 92.70% of these dogs are domiciliated (ALVES et al., 2005). Therefore, the initial population of pet dogs in Jataizinho, PR, was estimated at 3,079 animals.

The calculation of the sample size was performed using the software Epi 6.0 (DEAN et al., 1994), with an expected prevalence of 50%, accuracy of 5% and level of confidence of 95%. The size of the sample obtained was multiplied by 1.5 (design effect – Deff) as a way of correcting for cluster sampling, totalizing 513 dogs.

Sampling

A 20% loss in visitation was estimated considering dogs younger than six months, dogs with aggressive behavior, refusal of the owner to participate in the study and closed houses. Six hundred and twenty households were visited, which were distributed into 42 randomly selected blocks, proportional to the number of households in each region and based on 15 households per block.

Sample collection

Sample collection was performed between July and November 2010, and an informed consent form was signed by the animal's owner before each collection.

A household epidemiological questionnaire was administered to the owners that contained the following variables: accumulation of rainwater near or in the yard; sewage and garbage disposal; vacant land; flooded areas; presence of rivers, lakes or forests near the household; observation of rats in the household; and presence of a toilet and debris outside the house. The following variables were investigated for each dog: gender; age; breed; vaccination history; contact with other dogs in the household or stray dogs, rodents, cattle or possums; access to the street; type of access, alone or accompanied by the owner; and hunting habits. A blood sample was collected from each dog to obtain serum samples, which were placed in sterile flasks, labeled and stored at –20°C until processing.

Microscopic agglutination test (MAT)

All of the serum samples were tested for anti-*Leptospira* spp. antibodies against 23 reference serovars: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Fortbragg, Whitcombi, Cynopteri, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Sentot and Ballum.

The cultures used as antigens were kept at 28°C for 5 to 10 days in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (DIFCO® – USA) modified by the addition of rabbit serum (FAINE, 1982). The sera were considered to be reactive when at least 50% of leptospire were agglutinated, starting at a titer of 100 and geometrically diluting by two for the determination of the maximum positive dilution (FAINE et al., 1999). The serovar presenting the highest agglutinating titer was considered to be the most likely serovar (VASCONCELLOS et al., 1997). Only the sera presenting coagglutination in the highest dilution were considered to be reactive to *Leptospira interrogans* (ALMEIDA et al., 1994).

Statistical analysis

The data obtained from the epidemiological questionnaire and the results of serodiagnosis were analyzed with the software EpiInfo (DEAN et al., 1994). The Chi-squared test or Fisher's Exact Test were used to determine the statistical significance of the data. The strength of the association was determined by calculating the odds ratio (95%

confidence interval). A 5% level of significance was adopted.

Results

Blood samples were collected from 653 dogs in 369 households distributed into five regions of the urban area of the municipality of Jataizinho, PR.

Among the 132/653 (20.21%) reactive samples, 15/132 (11.36%) were reactive for more than one serotype, and the most likely serovar could not be determined; whereas of the 117/132 (88.73%) samples that reacted to a single serovar, Canicola was the most likely serovar in 89 (76.07%) samples.

The most likely serovars detected were Canicola, Butembo, Pyrogenes, Grippytyphosa, Ballum, Bratislava, Copenhageni, Pomona and Tarassovi, with titers varying between 100 and 12.800 (Table 1).

The significant variables ($p \leq 0.05$) associated with seropositivity for leptospirosis in dogs were the presence of rats in the household, adult or elderly dogs, free access to the street and contact with stray dogs (Table 2).

Table 1. The most likely serovars of *Leptospira interrogans* and the titers detected in the microscopic agglutination test (MAT) in 117 pet dogs in the urban area of Jataizinho, PR, between July and November 2010.

Titers	100	200	400	800	1600	3200	6400	12800	Total (%)
Canicola	30	28	14	09	03	03	01	01	89 (76.07)
Butembo	02	02	01	02					07 (5.99)
Pyrogenes	05								05 (4.27)
Grippytyphosa	03	02							05 (4.27)
Ballum	03		01						04 (3.42)
Bratislava	03								03 (2.57)
Copenhageni	01			01					02 (1.69)
Pomona	01								01 (0.85)
Tarassovi	01								01 (0.85)
Total	49	32	16	12	03	03	01	01	117
(%)	(41.89)	(27.35)	(13.67)	(10.26)	(2.57)	(2.57)	(0.85)	(0.85)	(100.00)

Source: Elaboration of the authors.

Table 2. Analysis of the variables associated with the risk of infection by *Leptospira* spp. evaluated in 653 pet dogs in the urban area of the municipality of Jataizinho, PR, between July and November 2010.

	Reactive/Total (%)	OR (95%CI)	P
Observation of rats in the household			
Yes	107/482 (22.2)	1.65	0.047
No	25/170 (14.7)	(1.02-2.66)	
Contact with stray dogs			
Yes	81/338 (24.0)	1.63	0.017
No	51/315 (16.2)	(1.10-2.41)	
Age			
Puppy (a)	9/80 (10.0)	2.3	0.0308
Adult (b)	96/4371 (20.6)	(1.11 – 4.74)	
Elderly (b)	27/70 (27.8)	3.42 (1.51 – 7.78)	0.0041
Type of access			
Restrict (c)	8/61 (13.1)	1.53	0.0429
Partial (c)	28/149 (18.8)	(0.65 – 3.58)	
Free (d)	43/137 (31.4)	3.03 (1.32 – 6.92)	0.0111

OR = odds ratio, CI = confidence interval and p = probability. The variables followed by letters in the columns differ significantly (p<0.05).

Source: Elaboration of the authors.

No significant differences were noted among the following variables: accumulation of rain water near the house (p=0.081); accumulation of rain water inside the house (p=0.307); flooded areas near the house (p=0.770); rivers or lakes near the house (p=0.800); forest regions near the house (p=0.470); vacant land near the house (p=0.773); sewage disposal (p=0.071); garbage disposal (p=0.477); presence of debris inside the house (p=0.621); presence of a toilet outside the house (p=0.053); gender (p=0.056); breed (p=0.197); contact with dogs inside the house (p=0.463); contact with rodents (p=0.083); contact with cattle (p=0.503); contact with possums (p=0.251); access to the street (p=0.102); or hunting habits (p=0.233).

Discussion

The majority of studies on the seroprevalence of canine leptospirosis in dogs considered to be domiciled indicate rates of infection below 18.0% in different states of Brazil (FÁVERO et al., 2002; MASCOLLI et al., 2002; LOPES et al.,

2005; MODOLO et al., 2006; MAGALHÃES et al., 2007; SILVA et al., 2009; SPOHR, 2009; MORIKAWA, 2010; GONÇALVES et al., 2010), whereas in stray dogs, a seroprevalence of 21.6% has been described in São Paulo, São Paulo state (SP) (YASUDA; SANTA ROSA; YANAGUITA, 1980), a seroprevalence of 21.2% in Londrina, PR (BENITEZ et al., 2010) and a seroprevalence of 20.0% in Patos, Paraíba (PB) (BATISTA et al., 2004). In the present study, the prevalence of anti-leptospiral antibodies in pet dogs (20.2%) was similar to that described in stray dogs and can be related to the free access of the studied dogs to the street and the frequency of contact with stray dogs or places that could be contaminated with leptospires.

The detection of the serovar Canicola as the most likely in 89 (76.07%) of the seroreactive dogs in the urban area of the municipality of Jataizinho, PR, is consistent with the results described by Rubel et al. (1997), who indicated the free access of dogs to the street as the most important risk factor for canine leptospirosis, particularly for the serovar Canicola.

Dogs are considered to be the main reservoirs of the serovar Canicola, and as asymptomatic carriers, they can eliminate bacteria in the urine over long periods without showing signs of infection (FAINE et al., 1999). Direct contact between healthy dogs and dogs that are asymptomatic carriers of leptospires can favor the transmission of *Leptospira* spp., given the species' behavior of smelling the urine of other animals and licking the genitals of females in heat. These behaviors may have been responsible for the high prevalence of dogs with antibodies against the serovar Canicola found in this study.

Of the 89 pet dogs that were found to be reactive to the serovar Canicola, 61 (68.54%) had direct contact with stray dogs. Access to the street without supervision was reported by the owners of 53 dogs, whereas eight other dogs, though kept under restraint in the backyards of their homes, had direct contact with stray dogs inside the household due to the lack of protective walls or fences around the houses.

In Brazil, several authors who have studied pet dogs in urban areas have detected a higher prevalence of seroreactive animals among adult and elderly dogs and associated this finding with the greater period of exposure and frequency of contact with different routes of transmission, represented by places contaminated by *Leptospira* spp. and stray dogs with leptospiuria (MASCOLLI et al., 2002; MAGALHÃES et al., 2007; FRAGA, 2008). In the present study, the variable of adult or elderly dogs was associated with dogs that were seroreactive in the MAT, which emphasizes the need to prevent the contact of pet dogs with stray dogs by controlling their access to the street.

Antibodies against the serovar Canicola can be detected after vaccination; however, a lack of vaccination against leptospirosis was reported in 100% of the dogs, which excluded the presence of vaccine antibodies. The high prevalence observed for this serovar was likely caused by exposure of these animals to leptospires in the environment and urine of infected dogs, which suggests a high

circulation of this serovar and serves as an alert for the utilization of antileptospirosis vaccines in the canine population of the studied area.

The presence of rodents was reported in 73.9% of households and was considered to be a risk factor associated with the seroprevalence of dogs with anti-leptospirosis antibodies. The presence of these rodents, described by the residents as mice, which could be both *Mus musculus* and/or *Rattus rattus*, could influence the prevalence of dogs' reactivity to the serovar Ballum; however, the habit of hunting rodents was not described for the dogs included in the study, limiting their direct contact with these animals. It is possible that the lack of dog owner observation could influence this variable. The dogs' indirect contact with the urine of rats was likely small, which may explain the low prevalence (3.4%) observed for this serovar.

In contrast to the majority of studies conducted on dogs in urban areas (VIEGAS et al., 2001; QUERINO et al., 2003; SILVA et al., 2009; MORIKAWA, 2010), the serovar Icterohaemorrhagiae was not detected in the dogs included in the present study. Given the residents' reports on the rodents observed in the household, this result would be expected, as according to Faine et al. (1999), the main reservoir of this serovar in urban areas is the sewer rat (*Rattus norvegicus*). Spohr (2009) detected no antibodies against the serovar Icterohaemorrhagiae in 172 rodents captured in 25 locations used for recycling collected solid residues and old iron in Londrina, PR. All of the captured rodents were identified as *Rattus rattus*, which is not considered to be a reservoir of the serovar Icterohaemorrhagiae.

The lower frequency of seroreactive dogs for the serovars Butembo (5.9%), Pyrogenes (4.2%), Grippotyphosa (4.2%) and Bratislava (2.5%) suggest the minimal contact of these dogs with reservoirs for these serovars. Similar results were detected by Lilenbaum, Rodrigues and Barboza (2000) when evaluating the relationship between different serovars of leptospires in the urban canine population of Oriximiná, Pará (PA), and the local

wildlife. These authors concluded that the possibility of contact with these animals was not decisive in the epidemiology of canine leptospirosis.

According to Masculli et al. (2002), the variants that circulate in the human and dog populations are the same, given that the proximity of these populations leads to their exposure to the same risk factors. During the period of study on dogs in the municipality of Jataizinho, PR, the serovar Canicola was detected by MAT in one human patient who had symptoms of chronic renal failure (*verbal communication). This result, associated with the high frequency of antibodies detected for serovar Canicola in the dogs included in the study, suggests the participation of pet dogs in the transmission of leptospirosis to humans in this municipality.

Conclusion

The high prevalence of pet dogs in the urban area of Jataizinho, PR, with anti-leptospiral antibodies, especially against serovar Canicola, indicates the need to adopt measures that will prevent the contact between pet and stray dogs, with the aim of interrupting the transmission cycle of these bacteria. The vaccination of dogs as well as owners knowledge of the epidemiological role of these animals in this important zoonosis is also essential.

Ethics committee

Approved by the Animal Experimentation Ethics Committee of the State University of Londrina, PR (CEEA/UEL), under protocol number 32/10.

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References

- ALMEIDA, L. P.; MARTINS, L. F. da S.; BROD, C. S.; GERMANO, P. M. L. Levantamento soropidemiológico de Leptospirase em trabalhadores do serviço de saneamento ambiental em localidade urbana da região sul do Brasil. *Revista de Saúde Pública*, v. 28, n. 1, p. 76-81, 1994.
- ALVES, M. C. G. P.; MATOS, M. R.; REICHMANN, M. L.; DOMINGUEZ, M. H. Dimensionamento da população de cães e gatos do interior do Estado de São Paulo. *Revista de Saúde Pública*, v. 39, n. 6, p. 891-897, 2005.
- BATISTA, C. S. A.; AZEVEDO, S. S.; ALVES, C. J.; VASCONCELLOS, S. A.; MORAIS, Z. M.; CLEMENTINO, I. J. LIMA, F. S.; ARAÚJO NETO, J. O. Soroprevalência para leptospirase em cães errantes da cidade de Patos, Estado da Paraíba, Brasil. *Brazilian Journal of Veterinary Research and Animal Science*, v. 41, n. 2, p. 131-136, 2004.
- BENITEZ, A.; RODRIGUES, G. G.; GONÇALVES, D. D.; BURKE, J. C.; ALVES, L. A.; MÜLLER, E. E.; FREITAS, J. C. Leptospirase em cães errantes encontrados em campus universitário: avaliação sorológica e exame direto da urina. *Semina: Ciências Agrárias*, Londrina, v. 31, n. 1, p. 191-196, 2010.
- BROD, C. S.; ALEIXO, J.A.G.; JOUGLARD, S. D. D.; FERNANDES, C. P. H.; TEIXEIRA, J. L. R.; DELLAGOSTIN, O. A. Evidence of dog as a reservoir for human leptospirosis: a serovar isolation, molecular characterization and its use in a serological survey. *Revista da Sociedade Brasileira de Medicina Tropical*, Uberaba, v. 38, n. 4, p. 294-300, jul./ago. 2005.
- DEAN, A. G.; DEAN, J. A.; COULOMBIER, D.; BRENDEL, K. A.; SMITH, D. C.; BURTON, A. H.; DICKER, R. C.; SULLIVAN, K.; FAGAN, R. F.; ARNER, T. G. Epi Info, Version 6: a word processing, data bases, and statistic program for epidemiology on microcomputers. Atlanta, Georgia: *Center for Diseases Control and Prevention*, 1994.
- FAINE, S. *Guidelines for the control of leptospires*. 2. ed Geneva: World Health Organization. 1982. (WHO offset publication, 67).
- FAINE, S.; ADLER, B.; BOLIN, C.; PEROLAT, P. *Leptospira and leptospirosis*. 2. ed. Melbourne: Medisci, 1999. 272 p.

- FÁVERO, A. C. M.; PINHEIRO, S. R.; VASCONCELLOS, S. A.; MORAIS, Z. M.; FERREIRA, F.; FERREIRA NETO, J. S. Most frequent serovars of leptospires in serological tests of buffaloes, sheeps, goats, horses, swines and dogs from several brazilian states. *Ciência Rural*, Santa Maria, v. 32, n. 4, p. 613-619, 2002.
- FRAGA, D. B. M. *Avaliação do apel do cão como reservatório na transmissão da leptospirose humana. Bahia*. 2008. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) – Fundação Oswaldo Cruz. Centro de Pesquisa Gonçalo Moniz, Salvador.
- GONÇALVES, C. C.; PAES, A. C.; LANGONI, H.; SILVA, R. C. da; GRECA, H.; CAMOSSO, L. G.; GUIMARÃES, F. F.; ULLMANN, L. S. Anticorpos para *Leptospira* spp., *Toxoplasma gondii* e *Neospora caninum* em cães errantes albergados em canil privado. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 62, n. 4, p. 1011-1014, 2010.
- HARKIN, K. R.; ROSHTO, Y. M.; SULLIVAN, J. T.; PURVIS, T. J.; CHENGAPPA, M. M. Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of revalence of urinary shedding of leptospires in dogs. *Journal of the American Veterinary Medical Association*, New York, v. 222, n. 9, p. 1230-1233, 2003.
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE. *Censo demográfico*. 2010. Disponível em: <www.ibge.gov.br/cidadesat/topwindow.htm?1>. Acesso em: 27 dez. 2011.
- JANSEN, A.; SCHÖNEBERG, I.; FRANK, C.; ALPERS, K.; SCHNEIDER, T.; KLAUS, S. Leptospirosis in Germany, 1962-2003. *Emerging Infectious Disease*, Atlanta, Georgia, v. 11, n. 7, p. 1048-54, 2005.
- LILENBAUM, W.; RODRIGUES, F.; BARBOZA, F. Aglutininas antileptospiras em caninos do município amazônico de Oriximiná-Pará, Brasil. *Revista Brasileira de Ciência Veterinária*, Niterói, v. 7, n. 3, p. 133-135, set./dez. 2000.
- LOPES, A. L. S.; SILVA, W. B.; PADOVANI, C. R.; LANGONI, H.; MODOLO, J. R. Frequência sorológica antileptospírica em cães: sua correlação com roedores e fatores ambientais, em área territorial urbana. *Arquivos do Instituto Biológico*, São Paulo, v. 72, n. 3, p. 289-296, jul./set. 2005.
- MAGALHÃES, D. F.; SILVA, J. A.; MOREIRA, E. C.; WILKE, V. M. L.; NUNES, A. B. V.; HADDAD, J. P. A.; MENESES, J. N. C. Perfil dos cães sororreagentes para aglutininas anti-*Leptospira interrogans* em Belo Horizonte, Minas Gerais, 2001/2002. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 59, n. 5, p. 1326-1329, 2007.
- MASCOLLI, R.; PINHEIRO, S. R.; VASCONCELLOS, S. A.; FERREIRA, F.; MORAIS, Z. M.; PINTO, C. O.; SUCUPIRA, M. C. A.; DIAS, R. A.; MIRAGLIA, F.; CORTEZ, A.; COSTA, S. S. da; TABATA, R.; MARCONDES, A. G. Inquérito sorológico para leptospirose em cães do município de Santana de Parnaíba, São Paulo, utilizando a campanha de vacinação anti-rábica do ano de 1999. *Arquivos do Instituto Biológico*, São Paulo, v. 69, n. 2, p. 25-32, abr./jun. 2002.
- MILLER, M. D.; ANNIS, K. M.; LAPPIN, M. R.; LUNN, K. F. Variability in results of the microscopic agglutination test in dogs with clinical leptospirosis and dogs vaccinated against leptospirosis. *Journal of Veterinary Internal Medicine*, v. 25, n. 3, p. 426-432, 2011.
- MINKE, J. M.; BEY, R.; TRONEL, J. P.; LATOUR, S.; COLOMBET, G.; YVOREL, J.; CARIOU, C.; GUIOT, A. L.; COZETTE, V.; GUIGAL, P. M. Onset and duration of protective immunity against clinical disease and renal carriage in dogs provided by a bi-valent inactivated leptospirosis vaccine. *Veterinary Microbiology*, v. 137, p. 137-145, 2009.
- MODOLO, J. R.; LANGONI, H.; PADOVANI, C. R.; SHIMABUKURO, F. H.; MENDONÇA, A. O.; VICTORIA, C.; SILVA, W. B. Investigação soroepidemiológica de leptospirose canina na área territorial urbana de Botucatu, São Paulo, Brasil. *Brazilian Journal of Veterinary Research and Animal Science*, São Paulo, v. 43, n. 5, p. 598-604, 2006.
- MORIKAWA, V. M. *Estudo sorológico da infecção por Leptospira spp. em uma área de ocupação irregular e de alto risco para a doença em cães em Curitiba, PR*. 2010. Dissertação (Mestrado em Sanidade Animal e Medicina Veterinária Preventiva) – Universidade Federal do Paraná, Curitiba.
- PRESCOTT, J. F. Canine leptospirosis in Canada: a veterinarian's perspective. *Canadian Medical Association*, Canadá, v. 178, n. 4, p. 397-398, 2008.
- QUERINO, A. M. V.; DELBEM, A. C. B.; OLIVEIRA, R. C.; SILVA, F. G.; MÜLLER, E. E.; FREIRE, R. L.; FREITAS, J. C. Fatores de risco associados à leptospirose em cães do município de Londrina – PR. *Semina: Ciências Agrárias*, Londrina, v. 24, n. 1, p. 27-34, jan./jun. 2003.
- RUBEL, D.; SEIJO, A.; CERNIGOI, B.; VIALE, A.; WISNIVESKY-COLLI, C. *Leptospira interrogans* en una población canina del Gran Buenos Aires: variables asociadas con la seropositividad. *Revista Panamericana Salud Publica*, v. 2, n. 2, p. 102-106, 1997.

- SILVA, W. B.; SIMÕES, L. B.; PADOVANI, C. R.; LANGONI, H.; LOPES, A. L. S.; MODOLO, J. R. Inquérito sorológico e distribuição espacial da leptospirose canina em área territorial urbana da cidade de Botucatu, São Paulo. *Veterinária e Zootecnia*, Botucatu, v. 16, n. 4, p. 656-668, 2009.
- SPOHR, K. A. H. *Leptospirose em ratos urbanos em Londrina, Paraná*. 2009. Dissertação (Mestrado em Ciência Animal) – Universidade Estadual de Londrina, Londrina.
- TRUEBA, G.; ZAPATA, S.; MADRID, K.; CULLEN, P.; HAAKE, D. Cell aggregation: a mechanism of pathogenic *Leptospira* to survive in fresh water. Research article. *International Microbiology*, Madrid, v. 7, p. 35-40, 2004.
- VASCONCELLOS, S. A.; BARABRINI JUNIOR, O.; UMEHARA, O.; MORAIS, Z. M.; CORTEZ, A.; PINHEIRO, S. R.; FERREIRA, F.; FAVERO, A. C. M.; FERREIRA NETO, J. S. 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 no período de janeiro a abril de 1996. *Arquivos do Instituto Biológico*, São Paulo, v. 64, n. 2, p. 7-15, 1997.
- VIEGAS, S. A. R. A.; TAVARES, C. H. T.; OLIVEIRA, E. M.; DIAS, A. R.; SANTOS, M. de F. P. Investigação sorológica para leptospirose em cães errantes na cidade de Salvador – Bahia. *Revista Brasileira de Saúde e Produção Animal*, Bahia, v. 2, n. 1, p. 21-30, 2001.
- WARD, M. P. Seasonality of canine leptospirosis in the United States and Canada and its association with rainfall. *Preventive Veterinary Medicine*, v. 56, p. 203-213, 2002.
- YASUDA, P. H.; SANTA ROSA, C. A.; YANAGUITA, R. M. Variação sazonal na prevalência de leptospirose em cães de rua da cidade de São Paulo. *Revista de Saúde Pública*, São Paulo, v. 14, p. 589-596, 1980.

