

HIGH LEVEL OF SEROVAR COPENHAGENI DETECTION IN DOG SERA FROM PATROCÍNIO, MG, BRAZIL

ALTA FREQUÊNCIA DO SOROVAR COPENHAGENI DETECTADO EM CÃES DO MUNICÍPIO DE PATROCÍNIO, MG, BRASIL

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ABSTRACT: This study aimed to evaluate the occurrence of canine leptospirosis and the possible risk factors associated with the disease in the municipality of Patrocínio, MG, Brazil. A cross-sectional observational study was carried out from July through August 2017. The municipality was divided into four regions (north, south, east and west) and a predefined number of neighborhoods (25) were randomly sampled in each region. Samples of blood serum were collected from 241 domiciled male and female dogs of different breeds and ages. To investigate the risk factors for canine leptospirosis, the owners of the animals were asked to fill out an epidemiological questionnaire. The following factors were evaluated: breed, sex, age, presence of rodents, type of diet, access to the street, vaccination, presence of flooded areas, and educational level of the owners. Blood serum samples were evaluated by the Microscopic Agglutination Test (MAT), using a collection of 24 live antigens. Of the 241 dogs evaluated, 32 (13.2%) were reactive. The most frequent serovars were: Copenhageni (37.5%) and Canicola (21.8%), followed by Icterohaemorrhagiae and Grippityphosa (12.5%), Pomona, Tarassovi and Butembo (9.3%) and Hardjo (6.2%). The presence of canine leptospirosis was associated with purebred dogs (OR=0.3059 [95% CI: 0.1430 – 0.6547]) and vaccination (OR=2.581 [95% CI: 1.198 – 5.563]). It was concluded that some dogs in the municipality of Patrocínio, MG have anti-*Leptospira* spp. antibodies and that the serovars most frequently identified were Copenhageni (37.5%) and Canicola (21.8%). Pure breeds and vaccination were factors associated with the prevalence of infection.

KEYWORDS: *Canis lupus familiaris*. *Leptospira interrogans*. MAT. Risk factors

INTRODUCTION

Leptospirosis is an infectious and zoonotic disease often occurring in developing countries. The disease is caused by the bacterium *Leptospira interrogans*, a spirochete-like etiological agent that comprises 22 different species, Ten of these species belong to group I (*Leptospira interrogans*, *Leptospira kirschneri*, *Leptospira noguchii*, *Leptospira borgpetersenii*, *Leptospira alexanderi*, *Leptospira weilii*, *Leptospira santarosai*, *Leptospira kmetyi*, *Leptospira alstoni* and *Leptospira mayottensis*), five to group II (*Leptospira licerasiae*, *Leptospira wolffii*, *Leptospira fainei*, *Leptospira broomii* and *Leptospira inadai*) and seven to group III (*Leptospira idonii*, *Leptospira meyeri*, *Leptospira terpstrae*, *Leptospira biflexa*, *Leptospira vanthielii*, *Leptospira yanagawa* and *Leptospira wolbachii*). This disease affects mainly the renal proximal tubules, causing the patient to become a

chronic renal carrier who eliminates the bacterium through his urine (ADLER; MOCTEZUMA, 2010). Leptospirosis can be transmitted in two ways: through indirect contact with contaminated soil, water and food, or through direct contact with infected animals (ACHA; SZYFRES, 2001). Rats are an important source of infection for dogs, since their habit of sniffing around garbage dumps and construction debris put them in contact with rat urine (NÓBREGA, 2015). On the other hand, dogs are part of the daily life of humans, and are thus an important source of infection for humans, since these animals can be renal carriers of the disease, even if they are asymptomatic (BIRCHARD; SHERDING, 2008). According to Brasil (2016), zoonotic diseases should be prevented through health education, environmental management to control and eliminate reservoirs, and through animal vaccination. The aim of this study was to evaluate the seroprevalence of canine leptospirosis and the risk factors associated with this infection in the

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municipality of Patrocínio, state of Minas Gerais – MG, Brazil.

CONTENTS

A cross-sectional observational study was carried out during July and August 2017. The research was approved by the Animal Research Ethics Committee of the University Center of Patos de Minas – UNIPAM, under protocol no. 62/17.

The total population of dogs in the municipality of Patrocínio (MG) was estimated based on the human population of 88.648, according to the Brazilian Institute of Geography and Statistics for the year 2015. The ratio of dogs to humans was calculated based on a proportion of 1:6 (THRUSFIELD, 2004), which resulted in a total of 14,774 animals. The sample was calculated within a confidence interval of 95% and a variation of 5%. Recent surveys of canine leptospirosis in Brazil have revealed a prevalence ranging from 6.8% to 35.1% (FERNANDES et al., 2013; LANGONI et al., 2015); therefore, an average prevalence rate of 20% was expected, resulting in a total of 241 samples.

The animals of this study were male and female dogs of different breeds and ages, which appeared to be healthy. The blood samples were collected randomly in 25 neighborhoods of the municipality. The number of samples per neighborhood was estimated according to the number of dogs vaccinated per neighborhood during the 2016 Dog Rabies Vaccination Campaign, based on data provided by the municipality's Zoonosis Control Center (ZCC).

Blood samples were collected by venipuncture of the accessory cephalic using 3 mL syringes and a 25x7 needles. The samples were placed in tubes without anticoagulant and stored under refrigeration (2-8°C), after which they were centrifuged at 2500 x g to obtain blood serum. The serum samples were subjected the Microscopic Agglutination Test (MAT). A collection of 24 live antigens of *Leptospira* spp. were used, comprising the serogroup Icterohaemorrhagiae (serovars Copenhageni and Icterohaemorrhagiae, serogroup Hebdomadis (serovar Hebdomadis), serogroup Autumnalis (serovars Autumnalis and Bratislava), serogroup Pyrogenes (serovar Pyrogenes), serogroup Bataviae (serovar Bataviae), serogroup Grippotyphosa (serovar Grippotyphosa), serogroup Canicola (serovar Canicola), serogroup Australis (serovars Australis and Butembo), serogroup Pomona (serovar Pomona), serogroup Sejroe (serovars Hardjo and Wolffii), serogroup Tarassovi

(serovar Tarassovi), serogroup Javanica (serovar Javanica), serogroup Panama (serovar Panama), serogroup Cynopteri (serovar Cynopteri), serogroup Semarang (serovar Patoc), serogroup Celledoni (serovar Whitcomb) serogroup Andamana (serovar Andamana), serogroup Ballum (serovar Castellonis), serogroup Shermani (serovar Shermani) and serogroup Djasiman (serovar Sentot). The tests were performed at the Laboratory of Leptospirosis and Brucellosis of UNESP at Jaboticabal, state of São Paulo.

The dogs' owners answered a questionnaire to determine the risk factors associated with canine leptospirosis. The following variables were investigated: breed, sex, age, access to the street, presence of rodents, existence of vacant lots near the home, the animal's diet, occurrence of floods, history of vaccination against leptospirosis, and the owners' level of education.

To calculate the frequency, the ratio of seroreactive dogs to the total number of sampled animals was established based on absolute and relative frequency, using descriptive statistical analysis. The association between the epidemiological variables and positive animals was determined by means of the Odds Ratio nonparametric test for two independent samples, at a significance level of 5%, using GraphPad version 5.0 for Windows (San Diego, California, USA).

Of the 241 tested dog serum samples, 32 (13.2%) were reactive. The most frequent serovar was Copenhageni (37.5%) followed by Canicola (21.8%) Icterohaemorrhagiae and Grippotyphosa (12.5%), Pomona, Tarassovi and Butembo (9.3%), and Hardjo (6.2%). The serotype with the highest identified titer was Butembo (3200). The titers ranged from 100 to 3200. Animals reactive to more than one serovar and which presented similar titers were considered positive for all the serovars, counted individually (Table 1).

Table 1. Seroprevalence and antibody titers to *Leptospira* spp. serovars in the Microscopic Agglutination Test performed on dogs of the municipality of Patrocínio, MG, Brazil, 2017.

Serovars	No.	%	Titers				
			100	200	400	800	3200
Copenhageni	8	25	4	2	2	-	-
Canicola	7	21.8	7	-	-	-	-
Butembo	3	9.3	2	-	-	-	1
Tarassovi	2	6.2	2	-	-	-	-
Icterohaemorrhagiae	2	6.2	2	-	-	-	-
Hardjo	2	6.2	1	1	-	-	-
Pomona	2	6.2	1	1	-	-	-
Grippotyphosa	1	3.2	1	-	-	-	-
Icterohaemorrhagiae/ Copenhageni*	2	6.2	1	-	1	-	-
Grippotyphosa/ Tarassovi*	1	3.2	1	-	-	-	-
Pomona/ Copenhageni/ Grippotyphosa*	1	3.2	-	1	-	-	-
Copenhageni/Grippotyphosa*	1	3.2	1	-	-	-	-
TOTAL	32	100	23	5	3	0	1

No.: number of reactive animals, * Animals reactive to more than one serovar with the same titration for both.

The prevalence rate found in this study (13.2%) was similar to the 13.1% found by Magalhães et al. (2007) in the city of Belo Horizonte, MG and lower than the 28.3% reported by Castro et al. (2015) in the city of Uberlândia, MG. Similar frequencies have been reported in other Brazilian states, such as 17.15% in Pará (PAZ et al., 2015), 19.7% in Paraíba (AZEVEDO et al. 2011) and 6.8% in Rio Grande do Norte (FERNANDES et al., 2013).

Most of the animals were reactive to the serovar Copenhageni (37.5%). Langoni et al. (2015) also reported this serovar as the one with the highest prevalence, while Fernandes et al. (2013) reported this serovar as the third most frequent. According to Mascilli et al. (2002), when the serovar Copenhageni is found to be the most prevalent, this indicates the influence of rodents, especially rats, as disseminators of the disease, since these animals are the main reservoirs of the disease. However, the presence of rodents was not identified as a risk factor for the occurrence of the infection ($P=0.2966$) in this study.

The serovar Canicola (21.8%) was the second most frequent. Castro et al. (2011) reported a similar finding in a study carried out in the city of Uberlândia, MG. Paz et al. (2015), Coiro et al. (2011) and Magalhães et al. (2007) also reported that the serovar Canicola appeared with the highest frequency. This serovar, whose natural hosts are dogs but which is also found in commercially available vaccines, causes the animal to present moderate clinical symptoms, but with a marked condition of leptospiremia, making it an important

source of infection for humans (HAGIWARA et al., 2004). In dogs, the renal carrier state is more pronounced in the presence of the serovar Canicola than the serovars found in the serogroup Icterohaemorrhagiae (MAGALHÃES et al., 2006).

About for the risk factors analyzed in the epidemiological questionnaire, the variables of breed ($OR=0.3059$ [95% CI: 0.1430 – 0.6547]) and vaccination ($OR=2.581$ [95% CI: 1.198 – 5.563]) were identified as increasing the chance of infection (Table 2).

Table 2. Distribution of dogs with anti-*Leptospira* spp. antibodies, according to the risk factors assessed, Patrocínio, MG, Brazil, 2017.

Variables	Dogs			OR*	95% CI	P
	Total	Reagents	Frequency (%)			
Level of education	149	16	10.06			
Elementary School (compl. or incomplete)	73	12	15.06			
Middle School (compl. or incomplete)	19	4	21.05	0.4511**	0.133 - 1.527	0.2494
Higher Education (compl. or incomplete)						
Age						
Puppy (< 1 year)	31	6	16.12			
Adult (1-8 years)	155	16	9.67	2.085**	0.743 - 5.844	0.2174
Elderly (> 8 years)	55	10	18.18			
Breed						
MBD	164	14	7.92	0.3059	0.143 - 0.65	0.0037
Purebred	77	18	22			
Sex						
Male	112	13	11.6	1.315	0.617 - 2.80	0.5692
Female	129	19	13.17			
Vaccinated						
Yes	102	20	18.62	2.581	1.198 - 5.56	0.0200
No	139	12	7.91			
Vaccinated < 1 year						
Yes	33	6	18.18	0.8730	0.301 - 2.52	1.0000
No	69	14	18.84			
Access to the street						
Yes	154	22	13.63	1.283	0.577 - 2.85	0.6931
No	87	10	10.34			
Empty lots						
Yes	111	13	9.90	0.7750	0.363 - 1.65	0.5706
No	130	19	14.61			
Rodents						
Yes	171	20	10.52	0.6402	0.294 - 1.39	0.2966
No	70	12	17.14			
Flooding						
Yes	42	7	14.28	1.810	0.715 - 4.57	0.2773
No	199	25	12.06			
Diet						
Commercial	158	27	15	1.855**	0.406 - 8.47	0.5367
Mixed	63	3	4.76			
Home cooked	20	2	10			

*Odds Ratio; **OR calculated for the greatest difference between proportions; MBD: mixed breed dog; p<0.05 indicates a significant difference.

Purebred animals presented a higher chance (OR=0.3059 [95% CI: 0.1430 - 0.6547]) of infection than mixed breed dogs. This finding contradicts that of Nóbrega (2015) for purebred dogs. A higher frequency of anti-*Leptospira* spp. antibodies was found among dogs of the Shih Tzu breed. This may be attributed to the fact that small

dog breeds are more popular today. Of the 77 purebreds evaluated here, only 17 were large dog breeds. Moreover, another factor possibly contributing to this finding is that these animals are often treated more carefully with respect to prevention measures, triggering vaccine reactions. Of the 18 purebred dogs that presented anti-

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Leptospira spp. antibodies, 15 had already been vaccinated. Two dogs tested positive for serovars Icterohaemorrhagiae and Copenhageni, which are part of the same serogroup Icterohaemorrhagiae. According to Costa (1996), this classification is based on their antigenic relationship, and their antigenic similarity enables the occurrence of cross-reactions. One of the two aforementioned dogs had not been vaccinated against leptospirosis, while the other one was vaccinated 10 months before its blood was sampled.

Vaccination history must be taken into account when interpreting results (OIE, 2014). Vaccines for leptospirosis provide immunity ranging from three to 12 months (RODRIGUES et al., 2013). Of all the animals that showed anti-*Leptospira* spp. antibodies, six had been vaccinated less than 12 months before their blood samples were collected. Among them, four had titers of 100, one had a titer of 200 for serovar Pomona, and one had a titer of 3,200 for serovar Butembo.

Titers of 100 can be interpreted as evidence of previous exposure to *Leptospira interrogans* or of vaccine reaction (OIE, 2014).

In this study, vaccinated animals had a higher chance of having the infection ($P=0.0200$), but when compared to the variable of time elapsed

from last vaccination, the latter showed no influence ($P=1.0000$). An absence of association can be explained by the fact that commercially available vaccines normally contain four serovars of the bacterium, i.e., Icterohaemorrhagiae, Canicola, Grippotyphosa and Pomona (TAVARES, 2015). Some vaccines also contain the serovars Copenhageni and Bratislava (FURQUIM, 2016), suggesting that vaccinated animals have not developed immunity or that the test was carried out within the vaccine's duration of protection, indicating that the positive result for anti-*Leptospira* spp. antibodies was due to vaccination.

The occurrence of leptospirosis was not associated with diet ($P=0.5367$), age ($P=0.2174$), sex ($P=0.5692$), level of education of the owners ($P=0.2494$), access to the street ($P=0.2773$), presence or absence of vacant lots near the residences ($P=0.5706$), and presence of rodents ($P=0.2966$).

It is concluded that dogs in the municipality of Patrocínio, MG, have anti-*Leptospira* spp. antibodies, and that the serovars Copenhageni (37.5%) and Canicola (21.8%) occurred most frequently. The risk factors associated with the prevalence of infection were the variables of being purebred and being vaccinated.

RESUMO: O objetivo deste estudo foi avaliar a ocorrência da leptospirose canina e os possíveis fatores de riscos associados à doença no município de Patrocínio – MG. Foi realizado um estudo observacional transversal, durante os meses de Julho à Agosto de 2017. O município foi dividido em quatro regiões (norte, sul, leste e oeste) e um número predefinido de bairros (25) foi amostrado aleatoriamente em cada região. Foram colhidas 241 amostras de soro sanguíneo de cães domiciliados de ambos os sexos e de diferentes raças e idades. Para investigação dos fatores de risco da leptospirose canina foi aplicado um questionário epidemiológico aos tutores dos animais, foram avaliados os fatores: raça, sexo, idade, presença de roedores, tipo de dieta, acesso à rua, vacinação, presença de áreas alagadas, terrenos baldios e grau de escolaridade dos tutores. As amostras de soro sanguíneo foram avaliadas pelo exame de Soroaglutinação Microscópica (SAM), com uma coleção de vinte e quatro antígenos vivos. Dos 241 cães avaliados, 32 (13,2%) apresentaram-se reagentes. Os sorovares de maior frequência foram: Copenhageni (37,5%) e Canicola (21,8%), seguido por Icterohaemorrhagiae e Grippotyphosa (12,5%), Pomona, Tarassovi e Butembo (9,3%) e Hardjo (6,2%). A presença de leptospirose canina foi associada em cães com raça definida (OR = 0,3059 [IC 95%: 0,1430 – 0,6547]) e vacinação (OR = 2,581 [IC 95%: 1.198 – 5.563]). Concluiu-se que existem cães que apresentam anticorpos anti-*Leptospira* spp., no município de Patrocínio-MG e que os sorovares Copenhageni (37,5%) e Canicola (21,8%) foram os de maior ocorrência. Apresentar raça definida e a vacinação foram fatores associados à prevalência da infecção.

PALAVRAS-CHAVE: *Canis lupus familiaris*. *Leptospira interrogans*. SAM. Fator de risco.

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