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Canine leptospirosis: an Overview of the City of Pelotas, Brazil

Leptospirose canina: uma visão da cidade de Pelotas, Brasil

Leptospirosis canina: una visión general de la ciudad de Pelotas, Brasil

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Abstract

Leptospirosis is a disease of worldwide importance, both from a veterinarian and a public health point of view. Serological survey through the microscopic agglutination test (MAT) is the standard to diagnose and assess the disease´s distribution in a population. Stray dogs are important urban reservoirs of leptospirosis and studies regarding their seroreactivity in Brazil are few and far apart. This work reports the seroreactivity of stray dogs to the most important serogroups in the *Leptospira* genus causing urban leptospirosis in dogs and humans in Pelotas, Brazil: Icterohaemorrhagiae and Canicola. All the animals used in this study were female stray dogs, no distinction regarding age or race was made. Blood samples were collected from 221 animals. The MAT was carried out according to the recommendations of the World Health Organization (WHO). Of the 221 tested animals, 64 were positive for agglutinating antibodies, representing a prevalence of 29%. These results are in accordance with those reported for housed dogs in Pelotas in previous studies. This study represents an important epidemiological update for the leptospirosis scenario in southern Brazil. Furthermore, these reports will aid healthcare agents in controlling both canine and human leptospirosis in the region.

Keywords: Canine leptospirosis; *Leptospira*; MAT.

Resumo

A leptospirose é uma doença de importância mundial, tanto para área veterinária quanto para a saúde pública. O teste de aglutinação microscópica (MAT) é o padrão para diagnosticar e avaliar a distribuição da doença em uma população. No Brasil, caninos não domiciliados são considerados importantes reservatórios urbanos da leptospirose, porém estudos sobre a prevalência da enfermidade são raros ou estão desatualizados. Assim, este trabalho relata a

sororeatividade de fêmeas caninas não domiciliadas para dois sorogrupos de *Leptospira interrogans*, importantes causadores da leptospirose urbana em caninos e humanos no Brasil e no mundo. Todos os animais usados neste estudo foram fêmeas, as quais eram pertencentes ao programa de controle da população canina da Prefeitura de Pelotas, sem a distinção de idade e raça. Amostras de sangue foram coletadas de 221 animais. O MAT foi realizado segundo as recomendações da Organização Mundial da Saúde (OMS). Dos 221 animais testados, 64 foram reagentes, representando uma prevalência de 29%. Esses resultados estão de acordo com os descritos em estudos anteriores para cães no município de Pelotas. Este estudo representa uma importante atualização epidemiológica para o cenário da leptospirose no sul do Brasil. Além disso, estes resultados ajudarão os agentes de saúde no controle da leptospirose canina e humana na região.

Palavras-chave: Leptospirose canina; *Leptospira*; MAT.

Resumen

La leptospirosis es una enfermedad de importancia mundial, tanto desde el punto de vista veterinario como de salud pública. El estudio serológico mediante la prueba de aglutinación microscópica (MAT) es el estándar para diagnosticar y evaluar la distribución de la enfermedad en una población. Los caninos callejeros son importantes reservorios urbanos de leptospirosis y los estudios sobre su serorreactividad en Brasil son pocos y distantes. Este trabajo reporta la serorreactividad de perros callejeros a los serogrupos más importantes del género *Leptospira* que causan leptospirosis urbana en perros y humanos en Pelotas, Brasil. Todos los animales utilizados en este estudio fueron hembras de perros callejeros, no se hizo distinción en cuanto a edad o raza. Se recolectaron muestras de sangre de 221 animales, se utilizaron *Leptospira interrogans* serogrupo Icterohaemorrhagiae y serogrupo Canicola. El MAT se ejecutó de acuerdo con las recomendaciones de la Organización Mundial de la Salud (OMS). De los 221 animales probados, 64 fueron positivos para anticuerpos aglutinantes, lo que representa una prevalencia del 29%. Estos resultados concuerdan con los descritos para perros alojados en Pelotas en estudios previos. Este estudio representa una importante actualización epidemiológica para el escenario de la leptospirosis en el sur de Brasil. Además, estos informes ayudarán a los agentes de la salud a controlar la leptospirosis canina y humana en la región.

Palabras clave: Leptospirosis canina; *Leptospira*; MAT.

1. Introduction

Leptospirosis is a disease of worldwide importance, both from a veterinarian and a public health point of view (Ellis, 2015). Although it is a considered a cosmopolite disease, it has grater prevalence in underdeveloped and developing countries, mainly due to environmental factors, climate, and susceptible host diversity, both domestic and wild (Adler, 2015).

Canine leptospirosis has similar symptoms to the disease in humans, such as jaundice, muscle pain, vomiting and, ultimately, death (Rissi et al., 2014). Clinical manifestations may be acute, involving several organs such as kidneys and liver, leading to jaundice and hemorrhages in the more severe cases (Reagan et al., 2019). Chronic cases may lead to asymptomatic carrier animals, which may shed the bacteria in the environment for several months undetected (Furlanello et al., 2020). Leptospirosis is then maintained in the environment by these susceptible reservoir hosts, species that are adapted to determined serogroups, of which they rarely suffer the severe disease, becoming renal carriers and chronic shedders of the bacteria in the environment (André-Fontaine et al., 2018). Studies have revealed that dogs are a significant risk factor for human leptospirosis (Ricardo et al., 2020, Silva et al., 2017).

Canine leptospirosis has been studied to some extent in Brazil (Castro et al., 2015, Sales et al., 2020), however these studies have been few and far apart. In 2002, the isolation of a *Leptospira interrogans* strain of the Canicola serovar, revealed that, of 105 canine tested the same year, 55 (52.4%) were reactive in the MAT (Brod et al., 2005). Other studies carried out in the city of Pelotas both in rural and urban settings revealed prevalence's varying from 2.66% (Jouglard et al., 2000) to 34.8% (Ávila et al., 1998), respectively. Nearly twenty years have passed since the last studies on canine leptospirosis in southern Brazil were published (Brod et al., 2005), and new studies are required to maintain the surveillance on the disease.

This study was carried out with the intention of updating the current situation on canine leptospirosis in the city of Pelotas. Our results indicate canines as an important reservoir host for leptospirosis, once again shining a light on the need to control stray dog populations in urban settings.

2. Material and Methods

In this work, we carried out a cross-sectional observational study (Thrusfield, 2007) of a quantitative nature (Pereira et al., 2018). All the animals used in this study were female stray dogs, collected by the Pelotas administration, to be castrated, according to the municipal dog control program. No distinction towards age or race was made. All animals were manipulated in accordance with the guidelines and approval of the Federal University of Pelotas Ethics Committee in Animal Experimentation (Protocol number 5104).

Blood samples were drawn using a vacutainer apparatus, from the cephalic vein, prior to the ovarian-histerectomy surgery as part of the standard pre-operatory exam. The blood was immediately stored at 4 ºC for posterior serum separation. Serum was stored at -20 ºC until use. Blood samples were collected from 221 animals.

Leptospira strains used in this study were isolates obtained by our group, described by Silva et al., (2008), of the Canicola and Icterohaemorrhagiae serogroups. The strains were kept at 30 ºC culture in EMJH liquid media. All media used in this study had 10% commercial supplement (DIFCO) added.

The MAT was carried out according to the recommendations of the World Health Organization (WHO, 2003). Briefly, the leptospires were counted in a Petroff-Hausser chamber, and the concentration was adjusted to $1x10^8$ leptospires/mL. The serum was diluted 1:25 in sterile PBS for screening, it was then incubated at 30 ºC with the live antigen (50 µL diluted serum and 50 µL ~10^8 culture) for a final screening serum dilution of 1:50. After two hours the reaction was observed and 50% or more agglutination was considered positive. Positive sera were tittered, using serial dilutions from 1:50 to 1:3,200 and repeating the previous procedure. The live antigens were maintained as previously described (WHO, 2003).

3. Results and Discussion

Pelotas is a near sea level city, with high annual rainfall and relative humidity, which increases the risk of leptospirosis outbreaks. The city has a high number of stray dogs, and these may be acting as reservoirs, shedding the bacteria, and transmitting the disease to humans and other dogs and domestic animals. Serologic evaluation of the stray dogs generates necessary public health information.

Of the 221 tested animals, 38 were positive for the Icterohaemorrhagiae serogroup and 40 for the Canicola serogroup. Of these, 14 were positive for both antigens, results are shown

in Table 1.

Table 1. Distribution of animals according to seroreactivity in MAT.

	Icterohaemorrhagiae	Canicola	Both	Total
Reactive (n)	24	26	14	64
% of total	10.9	11.8	6.3	29
% of positive	37.5	40.6	21.9	100

Source: This study.

The total prevalence was of 29% positive dogs. Of these, 11.8% were positive for Canicola, 10.9% for Icterohaemorrhagiae, and 6.3% for both strains. Furthermore, titers varied from 50 to 3,200 (highest titer assayed). These results are shown in Table 2.

Table 2. Number of positive sera by titer.

	Icterohaemorrhagiae	%	Canicola	%	Total	%
1:50	21	55.3	20	50	41	52.6
1:100	7	18.4	3	7.5	10	12.8
1:200	6	15.8	3	7.5	9	11.5
1:400	2	5.3	8	20	10	12.8
1:800	1	2.6	2	5	3	3.9
1:1,600	1	2.6	2	5	3	3.9
1:3,200	0	0	2	5	2	2.6
Total	38	100	40	100	78	100

Source: This study.

In this study, we found the highest titers for serovar Canicola, while the highest number of reactions for serovar Icterohaemorrhagiae, with lower titers ranging from 50 to 200.

Previous studies have shown different serological prevalence in Pelotas. Ávila et al., (1998) found 34.8% prevalence in 425 animals tested, these result were similar to our 29% prevalence. Although they used six serovars in the MAT, 80% of the positive animals were reactive to the two serovars we used, therefore justifying our methods. However, their study did not discriminate housed and stray dogs, therefore many positive animals may have vaccine induced agglutinins.

Another study found 28.9% positivity (Furtado et al., 1997), indicating that little has changed since 1997, and the city's attempts to control the disease in stray dogs are, thus far, frustrated. In dogs from rural settings in the region of Pelotas the seroprevalence found was 2.66% (Jouglard et al., 2000). However the different setting is most likely responsible for the very different outcome. Most animals in our study reacted weakly to the MAT, especially with 1:50 titters. This is likely due to the fact that we used only two strains to screen the sera, and some cross reaction with untested serovars may be occurring. Our use of the two serogroups is justified by several authors who have described these as being the most prevalent serovars in canine populations in the world and in Pelotas (Ávila et al., 1998).

Of the 221 animals tested, 96 had a known neighborhood of origin. The results regarding these animals are presented in Table 3.

Table 3. Distribution of dogs positive in the MAT according to the neighborhood.

MAT	Neighborhood							Total
	Centro	Fragata	Três Vendas	Areal	Laranjal	São Gonçalo	Jardim América	
(+)	1	7	10	4	0	6	3	31
%	11.1	33.3	28.6	40.0	-	75.0	27.3	32.3
Total	9	21	35	10	2	8	11	96

Source: This study.

When the location of capture was considered, our highest seroprevalence was in the São Gonçalo region, this may be explained by the fact that it is a poor area of the city, with some slum settings. It is also a low region, two meters above sea level as opposed to the average seven meters of the rest of the city, with higher chances of flooding. This maintains terrain height as a risk factor for canine leptospirosis, agreeing with previous studies (Ávila et al., 1998).

4. Conclusion

The results described in this assay are of great concern regarding the strategies, or lack thereof, being undertaken to control leptospirosis in stray dogs, indicating that little has changed in the city of Pelotas in the past ten years. Prevalence and risk factors similar to those described all those years ago are still the same today. The authors urge the authorities to

practice effective combat strategies against canine leptospirosis in Pelotas, and call upon other researchers to monitor the disease in this, and other cities. Surveillance on canine leptospirosis should be recurrent, as these studies not only reveal the disease burden on dogs, but act as sentinels for the circulation of the bacteria in cities, and the risk for humans. Future studies should also consider this surveillance tool in housed dogs, as the present results regard only strays. Likewise, other serogroups could be included in future screenings, for a more ample epidemiological coverage.

Declaration of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of paper.

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