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Dissertação

**Pesquisa de anticorpos anti-*Leptospira* em cães e gatos na cidade de
Pelotas/RS**

Francieli Dell'Osbel

Pelotas, 2024

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Pelotas/RS**

Dissertação apresentada ao Programa de Pós-Graduação em Veterinária da Faculdade de Veterinária da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências (área de concentração: Saúde Única)

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À minha família, pelo amor, apoio e dedicação.

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***“Fiz a escalada da montanha da vida removendo pedras e plantando flores”
Cora Coralina***

Resumo

DELL'OSBEL, Francieli. **Pesquisa de anticorpos anti-*Leptospira* em cães e gatos na cidade de Pelotas/RS**. 2024. 51f. Dissertação (Mestrado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2024.

A leptospirose é endêmica nos principais centros urbanos do Brasil, afetando diversos mamíferos, inclusive humanos. Embora soroprevalências significativas tenham sido observadas em populações de gatos em todo o mundo, relatos clínicos e inquéritos sorológicos da leptospirose são relativamente raros no Brasil. Por outro lado, os caninos são considerados como importantes fontes de infecção para humanos, mesmo existindo vacinas comerciais para essa espécie. O diagnóstico sorológico da leptospirose é realizado através do teste de soraglutinação microscópica (MAT), considerado teste padrão ouro e apropriado para estudos epidemiológicos. Neste contexto, este trabalho teve como objetivo avaliar a soroprevalência de anticorpos anti-*Leptospira* em gatos e cães domiciliados em Pelotas, sul do Brasil. Amostras de sangue de 101 gatos e de 240 caninos foram utilizadas para a detecção de anticorpos anti-leptospiras. Informações sobre os animais, incluindo sexo, idade, raça e área de residência foram coletadas, indicando que todos os animais eram domiciliados. No estudo envolvendo gatos, a soroprevalência global foi de 13,8%. A frequência de animais sororeagentes por sexo foi de 64,3% para machos e 35,7% para fêmeas, porém sem diferença estatística entre os sexos ($p > 0,05$). Os sorogrupos mais prevalentes foram o Icterohaemorrhagiae, seguido por Canicola. Das 240 amostras de soro dos caninos, informações sobre os animais como o sexo, 151 fêmeas e 89 machos, idade, raça e bairro também foram disponibilizadas. Do total de soros, 56 testaram reagentes no MAT. Entre os sorovares mais prevalentes, Canicola com 51,7%, seguido por Grippotyphosa com 46,4%. Em relação ao sexo, dos 56 soros reagentes, 37 eram fêmeas e 19 eram machos. Dado o seu status de zoonose, considerando a relevante soroprevalência relatada neste estudo e devido a crescente preocupação da leptospirose urbana como uma doença negligenciada no contexto do One Health, conclui-se que estudos sorológicos e epidemiológicos devem ser desenhados para examinar populações de cães e gatos em bairros de Pelotas com problemas históricos de inundação e altas populações de roedores, a fim de determinar o real potencial desses animais em atuar como reservatórios da enfermidade no município.

Palavras-chave: Leptospirose; Doença Negligenciada; Saúde Única; Zoonose; Epidemiologia

Abstract

DELL'OSBEL, Francieli. **Serological Survey of Anti-*Leptospira* Antibodies in Dogs and Cats in the City of Pelotas/RS**. 2024. 51f. Dissertation (Master degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2024.

Leptospirosis is endemic in major urban centers in Brazil, affecting various mammals, including humans. Although significant seroprevalences have been observed in cat populations worldwide, clinical reports and serological surveys of leptospirosis are relatively rare in Brazil. On the other hand, canines are considered important sources of infection for humans, despite the existence of commercial vaccines for this species. The serological diagnosis of leptospirosis is performed through the microscopic agglutination test (MAT), considered the gold standard test and appropriate for epidemiological studies. In this context, this study aimed to evaluate the seroprevalence of anti-*Leptospira* antibodies in domiciled cats and dogs in Pelotas, southern Brazil. Blood samples from 101 cats and 240 dogs were used for the detection of anti-leptospiral antibodies. Information about the animals, including sex, age, breed, and area of residence, was collected, indicating that all animals were domiciled. In the cat study, the overall seroprevalence was 13.8%. The frequency of seropositive animals by sex was 64.3% (n=9; 95% CI = 38.3 - 83.6%) for males and 35.7% for females, with no statistical difference between sexes ($p > 0.05$). The most prevalent serogroups were Icterohaemorrhagiae followed by Canicola. From the 240 dog serum samples, information about the animals such as sex, 151 females and 89 males, age, breed, and neighborhood was also provided. Of the total sera, 56 tested positive in the MAT. Among the most prevalent serovars, Canicola accounted for 51.7%, followed by Grippityphosa with 46.4%. Regarding sex, of the 56 positive sera, 37 were females and 19 were males. Given its zoonotic status, considering the significant seroprevalence reported in this study and the increasing concern of urban leptospirosis as a neglected disease in the One Health context, it is concluded that serological and epidemiological studies should be designed to examine populations of dogs and cats in neighborhoods of Pelotas with historical flooding problems and high rodent populations, in order to determine the real potential of these animals to act as reservoirs of the disease in the municipality.

Keywords: Leptospirosis; Neglected disease; One health; Zoonosis; Epidemiology

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Lista de Abreviaturas e Siglas

CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
DFM	Microscopia Direta de Campo Escuro
EMJH	Ellinghausen-McCullough Johnson Harris
GEDTA	Grupo de Estudos de Doenças Transmitidas por Animais
MAT	Teste de Aglutinação Microscópica
PBS	Solução Salina Tamponada com Fosfato
PCR	Reação em Cadeia da Polimerase
RS	Rio Grande do Sul
UFPEL	Universidade Federal de Pelotas
WHO	<i>World Health Organization</i> (Organização Mundial da Saúde – OMS)

Lista de Símbolos

<	Menor
>	Maior
%	Percentual
°C	Graus Celsius

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1 Introdução

Zoonose emergente e de distribuição mundial, a leptospirose é causada por uma bactéria espiroqueta móvel, do gênero *Leptospira* (REAGAN & SYKES, 2019). A doença foi encontrada em quase todas as regiões do mundo, com exceção apenas das regiões polares e em todas as espécies animais examinadas por pesquisadores experientes no assunto (ELLIS, 2015). Todos os anos, mais de um milhão de casos de leptospirose são notificados em todo o mundo. A infecção em humanos é acidental e pode ocorrer por contato direto ou indireto com a urina de animais infectados (RICARDO; PREVITALI; SIGNORINI, 2020).

Tais bactérias, são caracterizadas por serem aeróbicas, finas e com pontas em formato de ganchos (REAGAN & SYKES, 2019). O gênero *Leptospira* apresenta um longo histórico de mudanças em sua classificação genômica e taxonômica, porém, atualmente, considera-se que tenha duas origens em comum, patogênica e saprófita (ORR et al.,2022).

Tradicionalmente a classificação das leptospiras era baseado em seu fenótipo antigênico, conhecidos mais de 250 sorovares patogênicos, classificados em 25 sorogrupos diferentes. Recentemente, uma abordagem molecular baseada na homologia do DNA, permitiu identificar espécies patogênicas de *Leptospira* as quais são os agentes mais comuns que causam a doença em animais selvagens e domésticos, e em humanos (GRIPPI et al, 2023).

As leptospiras não são capazes de se replicarem fora de um hospedeiro e são facilmente inativadas quando não estão em condições apropriadas, mesmo assim, os sorovares patogênicos podem se manter viáveis no solo e na água por semanas. A partir disso, um número grande e variável de mamíferos pode se infectar com os sorovares patogênicos através do contato com essas águas e solos. A doença é descrita tanto em cães quanto em gatos, porém, os gatos aparentam ser mais resistentes a ela (REAGAN & SYKES, 2019).

A transmissão da leptospirose pode ocorrer de formas variadas, incluindo contextos ligados a urbanização. Os roedores e os cães são os principais reservatórios de espiroquetas nessas áreas. Os cães que vivem vagando livremente

nas cidades se tornam extremamente vulneráveis a doença, assim como os cães de áreas rurais, os quais tem maior contato com espécies de vida selvagem, que também são capazes de transmitir a doença (AEDO & MONTI, 2022).

Devido ao contato direto e a convivência entre humanos e animais de estimação, como cães e gatos, existe uma preocupação relacionada a transmissão da leptospirose. Isso se observa já que os cães e gatos, por vezes, atuam como predadores de roedores, favorecendo desta forma um canal único de transmissão da leptospirose de roedores para humanos (ELLIS, 2015).

A determinação dos sorovares causadores da leptospirose em cães é algo complexo, pois a maioria dos estudos existentes relata achados sorológicos que podem não representar o sorovar infectante de fato. Os anticorpos normalmente identificados são para os sorovares Gryppotyphosa, Bratislava, Canicola, Icterohaemorrhagiae, Autumnalis e Pomona. Em cães, os sorovares que mais comumente desenvolvem a doença em cães são Canicola, Pomona, Bratislava, Icterohaemorrhagiae e Gryppotyphosa (REAGAN & SYKES, 2019). Já os gatos, podem ser infectados e podem ser hospedeiros acidentais de alguns sorovares de *Leptospira* que são prevalentes na vida selvagem ou em outros animais domésticos, como Ballum, Copenhageni, Hardjo, Icterohaemorrhagiae, Rachmati, Bratislava, Bataviae, Canicola, Autumnalis e Gryppotyphosa (AEDO; MONTI; JARA, 2014).

A infecção por *Leptospiras* ocorre frequentemente através das membranas mucosas dos olhos, boca, nariz ou trato genital, porém a infecção por via oral também já foi evidenciada em predadores. Após 1 ou 2 dias a partir da infecção, inicia-se um período de bacteremia, que pode durar em torno de uma semana. Durante este período, as leptospiras podem ser isoladas a partir do sangue, da maioria dos órgãos do corpo e também do líquido cefalorraquidiano. A fase da bacteremia primária termina com o aparecimento de anticorpos circulantes que são detectados normalmente após 10 a 14 dias. Depois do período de leptospiremia, as *leptospiras* passam a se localizar nos túbulos renais proximais, onde multiplicam-se e são eliminadas na urina (ELLIS, 2015).

A leptospirose canina varia de infecções assintomáticas e crônicas, até quadros clínicos agudos caracterizados por febre, anorexia, vômitos, icterícia severa, hemorragia sistêmica e pulmonar, lesões renais e hepáticas e podendo levar até a morte, dependendo da virulência de cada cepa.

Os sinais clínicos em gatos ainda são pouco elucidados, no entanto, tem sido relatados sinais como poliúria, polidipsia, hematúria, ascite e diarreia. A apresentação clínica da leptospirose em gatos é rara, sendo geralmente leve ou subclínica e os sinais clínicos, na maioria das vezes, são indefinidos (KAKITA et al, 2021).

O diagnóstico da leptospirose pode ser obtido através de métodos sorológicos e bacteriológicos. A resposta a partir de anticorpos é detectável apenas depois de dias ou semanas após o início da doença. Para tal, utiliza-se da microscopia direta de campo escuro (DFM), o isolamento das leptospirosas por cultura bacteriológica, da detecção de DNA genômico por métodos moleculares e através da detecção de anticorpos por testes sorológicos (FONTAINE, 2006). O método considerado padrão ouro no diagnóstico sorológico da leptospirose é o MAT. O teste usa diluições seriadas do soro, que reage com culturas vivas de leptospirosas. Para realização do MAT usa-se um painel de sorovares, os mais comumente associados à infecção de cada espécie a ser estudada (REAGAN & SYKES, 2019).

O tratamento da leptospirose se dá a partir de antibióticos para controle da infecção, normalmente usando-se a associação de penicilina e estreptomicina, mas tendo bons resultados com o uso de ampicilina, amoxicilina, tetraciclina, tulatromicina e cefalosporinas de terceira geração também. A fluidoterapia é empregada para controlar os desequilíbrios eletrolíticos, transfusões de sangue também podem ser necessárias, assim como a diálise em casos mais severos. Além disso, o tratamento sintomático da doença também é empregado (ELLIS, 2015).

Neste contexto, apresentamos o artigo 1, que foi submetido à revista Cadernos de Saúde Pública (CSP), o qual determinou a prevalência de anticorpos anti-*Leptospira* em uma amostra de gatos na cidade de Pelotas/RS. Em seguida, o artigo 2, estudo que traz o conhecimento acerca da prevalência de anticorpos anti-*Leptospira* em uma amostra de cães domiciliados da cidade de Pelotas/RS, o qual está em processo final de formatação para submissão.

2 Hipótese

Cães e gatos domiciliados na cidade de Pelotas apresentam uma prevalência de anticorpos anti-*Leptospira* igual ou superior à média do país, o que implica em um potencial risco de transmissão da doença para humanos e outros animais de Pelotas, Rio Grande do Sul.

3 Objetivos

3.1 Objetivo Geral

Este estudo foi realizado com o intuito de atualizar o status da leptospirose canina e felina na cidade de Pelotas/RS, através do teste de soroaglutinação microscópica (MAT).

3.2 Objetivos Específicos

- Realizar um levantamento sorológico em cães domiciliados em Pelotas para determinar a prevalência de anticorpos anti-*Leptospira*;
- Realizar um levantamento sorológico em gatos domiciliados em Pelotas para determinar a prevalência de anticorpos anti-*Leptospira*;
- Identificar os sorovares de *Leptospira* mais prevalentes em cães e gatos domiciliados em Pelotas;
- Avaliar a relação entre a presença de anticorpos anti-*Leptospira* em cães e gatos e fatores como sexo, idade, raça e bairro de residência.

4 Artigos

4.1 Artigo 1

Prevalence of *Leptospira* spp. in domiciled cats from Pelotas (RS) and its importance in One Health

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Artigo submetido à revista Cadernos de Saúde Pública (CSP)

Prevalência de *Leptospira* spp. em gatos domiciliados de Pelotas (RS) e sua importância na Saúde Única

Prevalence of *Leptospira* spp. in domiciled cats from Pelotas (RS) and its importance in One Health

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ABSTRACT

Leptospirosis is endemic among animals in Brazil, affecting various hosts, including humans. Clinical disease reports and serological surveys of leptospirosis in cats are relatively rare in Brazil. Therefore, this study aimed to assess the seroprevalence of anti-leptospiral antibodies in non-vaccinated domiciled cats in Pelotas, southern Brazil. Blood samples from 101 cats were tested by microscopic agglutination test (MAT) for the presence of antibodies against a panel of 11 live antigens, including three local isolates. The overall seroprevalence of 13.8% was observed in the MAT (titers of ≥ 100). The frequency of seroreactive animals by sex was 64.3% for males and 35.7% for females. Seroreactivity against serogroups was Icterohaemorrhagiae, followed by Canicola, Grippityphosa and Australis, for which a local isolate named Hook was used. All reactive cats were of mixed breed and lived in the seven neighborhoods of Pelotas city. Considering the high seroprevalence documented in this study and the growing concern of urban leptospirosis as a neglected disease in the context of One Health, longitudinal studies can be designed to examine cat populations in neighborhoods of Pelotas with historical flooding issues and known high rodent populations to determine the true potential of cats to act as reservoir hosts.

Leptospirosis; Neglected disease; One health

RESUMO

A leptospirose é endêmica entre os animais no Brasil, afetando diversos hospedeiros, incluindo humanos. Relatos da doença clínica e levantamentos sorológicos da leptospirose em gatos são relativamente raros no Brasil. Por isso, este estudo teve como objetivo avaliar a soroprevalência de anticorpos anti-*Leptospira* em gatos domiciliados não vacinados em Pelotas, sul do Brasil. Amostras de sangue de 101 gatos foram testadas pelo teste de aglutinação microscópica (MAT) para a presença de anticorpos contra um painel de 11 antígenos vivos, incluindo três isolados locais. Foi observada uma soroprevalência geral de 13,8% no MAT (títulos de ≥ 100). A frequência de animais sororreagentes por sexo foi de 64,3% para machos e 35,7% para fêmeas. A sororreatividade contra os sorogrupos foi Icterohaemorrhagiae, seguido por Canicola, Grippotyphosa e Australis, um isolado local. Todos os gatos reagentes eram de raça mista e viviam nos sete bairros da cidade de Pelotas. Considerando a alta soroprevalência documentada neste estudo e a crescente preocupação com a leptospirose urbana como uma doença negligenciada no contexto de saúde única, estudos longitudinais podem ser desenhados para examinar populações de gatos em bairros de Pelotas com problemas históricos de inundações e com altas populações de roedores, para determinar o verdadeiro potencial dos gatos como hospedeiros reservatórios da enfermidade.

Leptospirose; Doença negligenciada; Saúde Única

INTRODUCTION

Leptospirosis is a zoonotic disease that is distributed worldwide¹. In Brazil, cases of human leptospirosis are reported in all federal units, with a higher number of cases in the southern and southeastern regions. The disease has an average lethality of 9%. Among confirmed cases of leptospirosis, males aged 20 to 49 are among the most affected, although there is no gender or age predisposition to acquiring the infection².

In Brazil and worldwide, the role of *Leptospira* as a pathogen in cats is not fully understood. While domestic felines were previously considered resistant to leptospirosis, it is now known that they can serve as both asymptomatic and symptomatic reservoirs of these bacteria³. Although there are few studies in the literature regarding the disease in cats, these primarily focus on renal injury⁴.

Risk factors for the disease in cats include proximity to other infected animals such as canines, felines, and other wildlife, as well as outdoor living or residing in rural environments⁵. However, the precise role of cats in the transmission of *Leptospira* remains unknown, with some authors suggesting that their significance in maintaining the pathogen may be underestimated⁶.

Serologic evidence supports natural infection in cats, with seroprevalences ranging between 4% and 20%⁷. The predominant serogroup identified in dogs and cats worldwide is Canicola, which also poses zoonotic risks. Additionally, serovars from the Icterohaemorrhagiae serogroup remain significant incidental infections in both dogs and cats on a global scale⁸.

Given this, research focusing on *Leptospira* spp. in cats is crucial for understanding the maintenance and transmission of the bacteria within this species. Thus, the objective of this study was to assess the seroprevalence of anti-leptospiral antibodies in cats from Pelotas, located in southern Brazil.

MATERIAL AND METHODS

The study was carried out in the municipality of Pelotas/RS, Southern Brazil. A serological survey was based on 101 serum samples from non-vaccinated cats collected between March 2022 and December 2023. All animals were presented to veterinary clinics, specifically Prontolab Clinic, with or without a history and clinical signs associated with leptospirosis. Limited data such as name, gender, age, and place of residence were possibly obtained from the medical records. A sample size of at least 101 cats was estimated through power analysis using OpenEpi 3.01⁹, based on a leptospiral antibody prevalence of 7% in cats in Brazil^{10,11}, with a 95% confidence interval and a 1% margin of error.

Anti-*Leptospira* antibody detection was conducted using the microscopic agglutination test (MAT), following the recommendations¹², and employing a collection of 8 serovars (Canicola, Copenhageni, Icterohaemorrhagiae, Pomona, Pyrogenes, Butembo, Grippotyphosa, and Patoc) from different serogroups and three local isolates belonging to Canicola, Australis, and Autumnalis serogroup. Titers were determined as the highest serum dilution at which at least 50% of the agglutinated leptospire were observed for each antigen, and animals were considered reactive when they exhibited titers ≥ 100 , although any titer should be considered relevant since there is no practice of vaccinating these animals in Brazil.

All 101 cats resided within nine neighborhoods of Pelotas city, with 65 from Centro; Areal (n=15); Laranjal (n=6); Três Vendas (n=6); Fragata (n=4); São Gonçalo (n=2); Balsa (n=1); Pestano (n=1); and Porto (n=1). There were 52 males and 49 females. The mean age of the cats was 8 years (range 2 months–22 years), and the cats belonged to three different breeds (Persian, n=2; Siamese, n=2; and mixed breed, n=97).

RESULTS

Of the 101 animals sampled, 14 (13.8%; 95% CI = 8.4 - 21.9%) animals were seroreactive in the MAT (titers of ≥ 100). The frequency of seroreactive animals by se

was 64.3% (n=9) for males and 35.7% (n=5) for females, with no statistically significant difference between the sexes ($p > 0.05$). Of the 14 cats seroreactive in the MAT, the most likely seroreactive serogroups were Icterohaemorrhagiae (50%), followed by Canicola (35.7%), Grippotyphosa (7.1%), and Australis (7.1%), for which a local isolate named Hook was used. The titers ranged from 100 (n=11) to 200 (n=3). Additionally, our study revealed six cats reacting with lower titers than the pre-established cut-off, with two animals, one male and one female, reacting at a titer of 1:50 against the serogroups Canicola and Australis (strain Hook), respectively. Two males reacted with a titer of 1:25 against the serogroups Australis (Hook) and Canicola (Kito), while two females reacted against Icterohaemorrhagiae and Patoc I. All reactive cats were of mixed breed and living in the following neighborhoods: Areal (26.7%), Laranjal (50%), Centro (3%), São Gonçalo (100%), Balsa (100%), Pestano (100%), and Porto (100%).

DISCUSSION

This is the first study to report the evaluation of antibody prevalence of *Leptospira* in a representative sample of non-vaccinated domiciled cats from Pelotas, Southern Brazil. Here, antibodies were detected in 14 (13.8%) of the cats, using a cut-off titer of 1:100, according to previous epidemiological studies conducted in Brazil and worldwide. However, this differs from other studies that included a dilution of 1:50 or lower, thus allowing the detection of cats with very low levels of anti-*Leptospira* antibodies.

In our study, antibody detection was also used to investigate the exposure of the studied cats to *Leptospira* in the past. Therefore, a dilution of 1:25 was also considered appropriate, as cats seem to respond to infection with low antibody titers¹³. The reason for the low titers in cats tested in this study could be due to serovars not tested or cross-reactions with others that were tested. Thus, titers ranging from 25 to 200 were observed, increasing the prevalence of antibodies from 13.8% to 19.8% (n=20), revealing that a significantly larger number had contact with the agent at some point in their lives or could be in the early stages of infection. Additionally, since cats could have a short-term immune response with a rapid decline in titers, the detection

of any response to the agent at any moment becomes crucial for the treatment, epidemiology, and prevention of the disease¹⁴.

According to our results, the predominant serogroup that reacted with the cats was Icterohaemorrhagiae, followed by Canicola and Australis, suggesting the circulation of the agent in different breeding styles, where cats, dogs, and rodents are similarly exposed, especially in neighborhoods of the city where prevalence is high¹⁵. This may indicate that cats can play an important role in the epidemiology of leptospirosis in Pelotas, either as an additional reservoir or simply as sentinels for the risk of infection. Cats included in this study were not suspected to have leptospirosis and exhibited a wide range of other clinical suspicions. Limitations of this study include the use of convenience samples from a clinical blood bank, which restricted access to some relevant information and the analysis of risk factors¹⁶. Future studies should incorporate serology in combination with molecular tools, such as urine real-time PCR, in addition to attempts to isolate the agent.

In summary, domiciled cats are exposed to *Leptospira* with a seroprevalence of 13.9%. The predominant serogroup was Icterohaemorrhagiae, which is consistent with findings in dogs and other domestic animals suspected to have leptospirosis in Pelotas. The data raises concern related to the role of domestic cats in the leptospirosis transmission cycle, including from the zoonotic aspect. In this way, considering the high seroprevalence documented in this study and the growing concern of urban leptospirosis as a neglected disease in the context of One Health, longitudinal studies can be designed to examine cat populations in neighborhoods of Pelotas with historical flooding issues and known high rodent populations to determine the true potential of cats to act as reservoir hosts.

Authors' Contributions

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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4.2 Artigo 2

Seroprevalence of Leptospiral Antibodies among Dogs in Pelotas, Southern Brazil

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ABSTRACT

A worldwide zoonosis, leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira*. Rats act as reservoirs, typically asymptomatic, harboring the spirochete in their renal tubules. Domestic animals, such as dogs, serve as carriers, intermittently or continuously excreting *Leptospira* in urine, contaminating the environment. The most used method for diagnosing leptospirosis is the MAT, considered the gold standard test. In this study, 240 serum samples from dogs were utilized. Information regarding the animals, including sex, 151 females and 89 males, age, breed, and area of residence, was provided, indicating that all animals were domiciled. Out of the total sera, 56 tested positive in the MAT with a titer equal to or greater than 1:100. Among the most prevalent serovars, Canicola with 51.7% of the reactions, followed by Grippotyphosa with 46.4% and Icterohaemorrhagiae with 41.07%. The remaining serovars accounted for 21.4% of the reactions combined. Regarding gender classification, out of the 56 reactive sera, 37 belonged to females and 19 to males. Given its status as a zoonosis, ongoing concern regarding the transmission and, especially, the prevention of leptospirosis in canines is imperative to avoid its spread in urban centers and flood-prone areas like Pelotas.

Keywords: Leptospirosis; One Health; Neglected Disease; Serology; Epidemiology; Dogs.

INTRODUCTION

Zoonosis of worldwide occurrence, leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira*. This genus has 64 named species and serologically classified into more than 250 serovars (MACALUSO et al., 2022). When it comes to dogs, the serovars most associated with infection are *Icterohaemorrhagiae*, *Canicola*, *Grippityphosa*, *Pomona*, and *Bratislava* (VAN DE MAELE et al., 2008).

The disease can be observed in all mammals, including marsupials. Rats act as reservoirs, usually asymptomatic, harboring the spirochete in their renal tubules. Domestic animals, such as dogs, act as carriers, intermittently or continuously excreting *Leptospira* in urine, contaminating the environment (PINTO et al., 2022). For this reason, the occurrence of floods, high rainfall rates, and the presence of rodents contribute to the spread of leptospirosis. This happens mainly in urban areas, making the disease a problem in the context of One Health (CUNHA et al., 2022).

As a consequence of rapid urbanization and increasing urban poverty, there is an increase in shantytowns settlements in low- and middle-income countries. Due to the lack of sanitation in these communities, residents of shantytowns and marginalized areas are increasingly exposed and at risk of acquiring water and animal-borne diseases, such as leptospirosis (FELZEMBURGH et al., 2014).

According to Felzemburgh et al. (2014), in Brazil, over 10,000 cases of leptospirosis are reported each year, the vast majority of which are residents of urban slums and require hospitalization due to severe complications of Weil's disease and pulmonary hemorrhage syndrome associated with leptospirosis. The clinical signs of leptospirosis vary, ranging from an undifferentiated febrile illness to multiple organ failure involving renal and pulmonary manifestations. Additionally, elevated serum levels of creatinine, thrombocytopenia, leukocytosis, and hyperbilirubinemia may occur. (PINTO et al., 2022).

The laboratory diagnosis of leptospirosis involves several methods, including the isolation of leptospire by bacterial culture, genomic DNA detection using

molecular methods, and antibody detection through serological tests (Fontaine, 2006). The Microscopic Agglutination Test (MAT) is the most used method for serological diagnosis of the disease, which utilizes live antigens representing the most prevalent serogroups worldwide. However, while the test is suitable for population-based serological surveys to determine disease status, it has limitations in sensitivity for acute cases because the antibody response becomes detectable only days or weeks after the onset of the disease (ELLIS, 2015)

Previous studies conducted by Ávila et al. (1998) and Felix et al. (2020), which reported a prevalence of 34.8% and 29%, respectively, represent some of the limited research on canine leptospirosis in Pelotas (RS) in recent years. Therefore, the objective of this study was to assess the seroprevalence of anti-leptospiral antibodies in domiciled dogs in Pelotas, southern Brazil.

MATERIAL AND METHODS

In this study, 240 serum samples from dogs were utilized, obtained through a partnership with a veterinary clinical analysis laboratory in the city of Pelotas (RS), collected between March 2022 and December 2023. Information regarding the animals, such as gender (151 females and 89 males), age, breed, and residential area, was provided; hence, all animals were domiciled. Vaccination history was not accessible.

A sample size of at least 240 dogs was estimated through power analysis using OpenEpi 3.01 (DEAN et al., 2013), based in one media on a leptospiral antibody prevalence of 20% in dogs worldwide (AVILA et al., 1998; FELIX et al., 2020; AEDO & MONTI, 2022), with a 95% confidence interval and a 1% margin of error.

The samples were forwarded to the Laboratory of the Study Group on Animal-Transmitted Diseases (GEDTA) at the Veterinary School of UFPel, for performing the Microscopic Agglutination Test (MAT). The forwarded samples were centrifuged at 3,000 rpm for 5 minutes, in microtubes, identified, and kept at -20°C until the test was conducted.

MAT was carried out following the recommendations of the World Health Organization (WHO, 2003). The sera were diluted 1:50 in sterile PBS for screening, aiming to enable the detection of the maximum number of acute-phase reactions of

the disease. A panel containing 7 pathogenic serovars (Butembo, Canicola, Copenhageni, Grippotyphosa, Icterohaemorrhagiae, Patoc, Pomona, and Pyrogenes) and one saprophyte (Patoc 1). The antigen battery was selected based on articles and on the prevalence of leptospirosis in studies conducted by the group. The antigens used for the MAT were cultivated for 7 days at 29°C, in liquid Ellinghausen-McCullough Johnson Harris (EMJH) medium.

RESULTS

Of the 240 analyzed sera, 151 were from females (62.9%; IC 95% = 56.6 – 68.7%) and 89 sera were from males (37.1%; IC 95% = 31.2 – 43.3%). Out of the total sera, 56 (23.3%; IC 95% = 18.4 – 29.0%) tested positive in the MAT with a titer equal to or greater than 1:100 (Table 1). Among the most prevalent serovars, Canicola with 51.7% (n=29; IC 95% = 38.9 – 64.3%) of the reactions, followed by Grippotyphosa with 46.4% (n=26; IC 95% = 33.9 – 59.3%) and Icterohaemorrhagiae with 41.07% (n=23; IC 95% = 29.1 – 54.1%). The remaining serovars accounted for 21.4% (n=12; IC 95% = 12.7 – 33.8%) of the reactions combined.

Regarding gender classification, out of the 56 reactive sera, 37 (66.1%; IC 95% = 52.9 – 77.0%) belonged to females and 19 (33.9%; IC 95% = 52.9 – 77.0%) to males (Table 2). Among the 37 females studied, the highest prevalence of anti-*Leptospira* antibodies was 54% for the Grippotyphosa serovar, 48.6% for Canicola, 37.8% for Icterohaemorrhagiae, 16.2% for Copenhageni, 8.1% for Butembo, and 2.7% for Pyrogenes. Among the 19 reactive sera from males, the highest prevalence of anti-*Leptospira* antibodies was 52.6% for Canicola, 47.4% for Icterohaemorrhagiae, 31.6% for Grippotyphosa, 5.3% for Pomona, and Butembo.

Regarding the age of the studied dogs, out of the 56 reactive sera, 54 had the age identified. We opted to divide the 54 animals into three categories according to age range: young (0-6 months), adult (7 months - 7 years), and senior (>8 years). The adult category had the highest number of reactions with 54 reactive sera (with the highest numbers of reactions for CAN, GRI, and ICT), followed by senior with 33 reactions (predominantly of the same serovars as the adult category), and lastly the young category, with only 2 reactions (ICT and GRI) (Table 3).

Relating the age and sex of the animals (36 females and 18 males with provided ages), the highest number of reactions in females occurred in the age range between 7 months and 7 years, with *Grippytyphosa* and *Canicola* being the most prevalent serovars (Table 4). As for males, the panel of reactions was similar concerning age range, but with a higher number of reactions for *Canicola*, followed by *Icterohaemorrhagiae* (Table 5).

Regarding the area of residence of the studied animals, the name of the neighborhood was provided for 40 out of the 56 reactive sera. The neighborhoods in question were: Centro, Areal, São Gonçalo, Fragata, Três Vendas, Laranjal, and Navegantes. Of these, the highest number of reactions was found in the Centro neighborhood with 57.5%, with *Canicola*, *Icterohaemorrhagiae*, and *Grippytyphosa* being the most prevalent serovars. Following Centro, the Areal neighborhood had 35% of the reactions for the same serovars as the Centro neighborhood. The other neighborhoods together accounted for a total of 35% of the reactions, following the same pattern of reactive serovars (Table 6).

In the study, the breeds of all animals were also provided, totaling 16 breeds: Shih-tzu (n=6), Golden Retriever (n=1), Fox Paulistinha (n=1), Lhasa Apso (n=1), Yorkshire Terrier (n=4), Mixed Breed (n=26), Poodle (n=3), Cocker Spaniel (n=1), Schnauzer (n=1), Beagle (n=2), Dachshund (n=1), Pinscher (n=1), Maltese (n=1), Chihuahua (n=1), French Bulldog (n=3), and Pitbull (n=3). Regarding the prevalence of reactions for each breed, Mixed Breed animals had the highest number of reactions with 66.1% predominantly for *Canicola*, *Icterohaemorrhagiae*, and *Grippytyphosa*, a number that can be attributed to the majority of animals falling into this breed category (Table 7).

DISCUSSION

In this study, the prevalence found in dogs was 23.3%. As leptospirosis is a zoonosis, concern regarding transmission and, primarily, prevention of the disease in canines should be continuous to avoid its spread in large urban centers and flood-prone areas, such as in the city of Pelotas.

According to the study by Avila et al. (1998) also conducted in the city of Pelotas, out of 425 canine sera analyzed, 148 (34.8%) sera were reactive, with 58.1% for *canicola*, 20.9% for *icterohaemorrhagiae*, 11.4% for *Copenhageni*, 2.7% for

grippotyphosa and castellanis, and 1.4% for andamana, autumnalis, and pyrogenes. Another study that supports the results presented in the current study is the one by Félix et al. (2020), where the prevalence of anti-leptospiral antibodies was 29% out of 221 tested dogs also in Pelotas, with 11.8% positive for Canicola, 10.9% for Icterohaemorrhagiae, and 6.3% for both strains.

Regarding the neighborhood of origin of the dogs, in the study by Félix et al. (2020), the neighborhood with the highest concentration of reactive animals in the MAT was São Gonçalo, followed by the Areal neighborhood. In our study, perhaps due to the fact that the animals were domiciled rather than stray, the highest concentration of reactive animals was found in the city center, followed by, in agreement with the study by Félix et al. (2020), the Areal neighborhood.

Both the study by Avila et al. (1995) and the study by Félix et al. (2020) tested serum samples from dogs in Pelotas for *L. icterohaemorrhagiae* and *L. canicola*, obtaining results similar to those found in the present study. Thus, there is a similar scenario in different time periods, reaffirming the importance of vaccinating dogs in an attempt to prevent leptospirosis in the municipality of Pelotas.

The prevalence of leptospirosis varies according to geographic location, the presence of animal reservoirs, interactions between vulnerable animal species, and the environment in which they are found. These factors are determinants of leptospirosis transmission and infection, along with climate and ecological changes, deforestation, global warming, and urbanization, which allow wild species to come into contact with domestic animals and people (AEDO & MONTI, 2022).

Also in the southern region of the country, in Porto Alegre, another study with 142 domiciled dogs obtained a similar scenario to that found in the city of Pelotas. 18.3% (26 animals) were seropositive for *Leptospira* sp, with the most frequently found serovars being: *Icterohaemorrhagiae* (12); *Canicola* (7); *Pomona* (4); *Autumnalis* (4), *Copenhageni* (3), and *Pyrogenes* (2) (MESQUITA et al., 2017).

Regarding the sex and breed of the canines, working dogs, male, intact, that have contact with rural areas, have a higher risk of contracting leptospirosis (KLOSOWSKI & BOHN, 2022). In our study, the dog population was in an urban area, but specific information about the animals' specific location of stay was not obtained, whether they lived in apartments or houses with access to yards or lawns, close to or

away from green areas. Based on this assumption, small breed dogs that have access to yards or patios also have a risk of contracting the disease, due to the possibility of wild animals entering these areas and contaminating the environment or the water provided to the dogs (KLOSOWSKI & BOHN, 2022). In a study conducted by Stull et al. (2022), males have also been reported to be more susceptible to acquiring leptospirosis, as well as Mini Toy and Terrier breeds. In our study, no significant association was found between the sex and breed of the animals, similar to the study by Pratt, Conan, and Rajeev (2017).

In a study conducted in southern Chile, 706 canines from urban (406) and rural areas (300) were tested, among them, 86 tested positive for MAT. Of the 406 urban dogs, 55.7% were females and 12.8% tested positive. Of the 300 rural dogs, 27.3% were females, but the highest number of positive test animals was 13.4% and were males (AEDO & MONTI, 2022).

The interpretation of positive titers for leptospirosis should take into account previous exposure and the vaccination status of the animals. Vaccination can induce positive MAT results for both vaccine and non-vaccine serogroups. Vaccine antibody titers typically begin to decrease 4 months after vaccination, and it may take up to 1 year for them to become very low. Most dogs develop only modest increases in MAT titers after vaccination, but titers greater than or equal to 1:600 have been documented after vaccination (REAGAN & SYKES, 2019). In our study, we did not have information about the vaccination status of each dog, so it is not within the scope of this study to infer whether the titers found in the MAT were vaccine-induced or due to exposure to serovars.

With the results obtained in this study, new approaches can be planned for the development of novel diagnostic formats for the disease, as well as the use of local vaccines or intensification of vaccination campaigns for dogs using vaccines already available in the market.

Declaration of Competing Interest

Nothing to declare.

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Authors' Contributions

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

Ethical statement

All procedures with the animal samples, as well as the related data, were carefully handled and judiciously utilized. Due to the use of samples from a serum bank, without animal manipulation and sample collection in this study, there was no need for formal ethical approval to conduct the study.

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Table 1. Distribution of reactions from 56 canine sera in the MAT according to reactive serovars and coagglutinations.

Serovars	400	200	100
CANICOLA	2	2	25
GRIPPOTYPHOSA	1	2	23
ICTEROHAEMORRHAGIAE	1	3	19
COPENHAGENI	0	1	5
BUTEMBO	0	0	4
POMONA	0	0	1
PYROGENES	0	0	1
Total	4	8	78

Table 2. Distribution of reactive males and females in the MAT according to the analyzed serovars.

	ICT	COP	CAN	GRI	POM	PYR	BUT
Female	14	6	18	20	0	1	3
Male	9	0	10	6	1	0	1

Table 3. Distribution of age of reactive animals in the MAT with each serovar.

	ICT	COP	CAN	GRI	POM	PYR	BUT	Total
Young (0-6 months)	1	0	0	1	0	0	0	2
Adult (7 months - 7 years)	12	2	17	17	1	1	4	54
Senior (>8 years)	10	4	11	8	0	0	0	33

Table 4. Relationship between age of females and reactions with antigens.

	Young (0-6 months)	Adult (7 months- 7 years)	Senior (>8 years)
Canicola	0	11	7
Grippotyphosa	1	12	6
Icterohaemorrhagiae	1	7	6
Copenhageni	0	2	4
Butembo	0	3	0
Pyrogenes	0	1	0
Total	2	36	23

Table 5. Relationship between age of males and reactions with antigens.

	Young (0-6 months)	Adult (7 months- 7 years)	Senior (>8 years)
Canicola	0	6	4
Icterohaemorrhagiae	0	5	4
Grippotyphosa	0	3	2
Pomona	0	1	0
Butembo	0	1	0
Total	0	16	10

Table 6. Distribution of the location of 40 reactive animals in the MAT and respective serovars.

Serovars	Areal	Centro	Fragata	São Gonçalo	Laranjal	Três	
						Vendas	Navegantes
ICT	5	7	0	1	0	0	0
COP	0	1	1	0	0	0	0
CAN	5	8	1	2	1	1	1
GRI	3	7	2	2	1	1	0
BUT	1	0	0	0	0	0	0
Total	14	23	4	5	2	2	1

5 Considerações Finais

De acordo com o artigo 1, a amostra de gatos domiciliados em Pelotas e que estão expostos à leptospirose, teve uma soroprevalência de 13,9%. O sorogrupo predominante foi *Icterohaemorrhagiae*, o que é consistente com achados em cães e outros animais domésticos com suspeita de leptospirose em Pelotas. Os dados levantam preocupações relacionadas ao papel dos gatos domésticos no ciclo de transmissão da leptospirose, inclusive sob o aspecto zoonótico. Desta forma, considerando a alta soroprevalência documentada neste estudo e a crescente preocupação da leptospirose urbana como uma doença negligenciada no contexto do One Health, estudos longitudinais podem ser desenhados para examinar populações de gatos em bairros de Pelotas, com histórico de problemas envolvendo inundações e altas populações de roedores, para assim reafirmar o potencial risco dos gatos na transmissão da leptospirose e como hospedeiros reservatórios.

No estudo do artigo 2, a prevalência encontrada na amostra de cães é de 23,3%. Como a leptospirose é uma zoonose, a preocupação com a transmissão e com a prevenção da doença em caninos deve ser contínua, a fim de evitar sua propagação em grandes centros urbanos e áreas propensas a inundações, como na cidade de Pelotas. Com os resultados obtidos neste trabalho, novas abordagens podem ser planejadas para o desenvolvimento de novos formatos diagnósticos para a doença, como os testes rápidos, bem como a utilização de vacinas locais e a intensificação de campanhas de vacinação para cães utilizando vacinas já disponíveis no mercado.

Portanto, os resultados obtidos nesta dissertação afirmam que a leptospirose em Pelotas continua sendo uma preocupação no contexto da Saúde Única, tanto em cães quanto em gatos, e apontam para o desencadeamento de políticas públicas, a fim de prevenir a transmissão dessa zoonose negligenciada, evitando agravos para a saúde animal e o potencial risco de transmissão da leptospirose para humanos.

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