DOI: 10.1002/vms3.704

ORIGINAL ARTICLE

WILEY

Serological survey of anti-*Leptospira* spp. antibodies in individuals with animal hoarding disorder and their dogs in a major city of Southern Brazil

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Funding information
Araucária Foundation of Paraná, Grant/Award Number: 41,769; Coordination for the Improvement of Higher Education Personnel of Brazil (CAPES)

Abstract

Background: Individuals with hoarding disorder (HD) presented a persistent difficulty in detaching from objects and/or animals. Unhealthy conditions, frequently found in cases of animal HD (AHD), may favour environmental contamination and the spread of zoonotic pathogens. Despite that, only one study of zoonotic diseases in individuals with AHD and their companion animals has been conducted to date.

Objectives: This study aimed to assess the seroprevalence of anti-*Leptospira* spp. antibodies in individuals with AHD and their dogs in a major city of Southern Brazil.

Methods: Blood samples were obtained from 264 dogs (21 households) and 19 individuals with AHD (11 households) and tested by microscopic agglutination test.

Results: All human samples were seronegative. Seropositivity was found in 16/264 (6.1%; CI 95% 3.3–9.6%) dogs from 11/21 (52.38%) households, with titres ranging from 100 up to 400, and Copenhageni (10/16; 62.5%) was the most frequent serovar. Surprisingly, seropositivity of hoarded dogs found herein was among the

lowest reportedly observed in other dog populations of Brazil. Two epidemiological variables were significantly associated with seropositivity in dogs: the presence of cat hoarding ($p = 0.004$) and the report of flood occurrence in the household ($p = 0.031$). **Conclusions:** No individuals with AHD were seropositive, and besides the lower seroprevalence of dogs, they probably had contact with *Leptospira* spp. at some point in their life. Since dogs can be considered potential sentinels in leptospirosis, public health programs must become aware of the risk of leptospirosis cases in households of individuals with AHD and nearby communities.

KEYWORDS
hoarding disorder, leptospirosis, One Health, sentinel surveillance

1 | INTRODUCTION

Hoarding is a psychological disorder defined as a persistent difficulty individuals have to discard objects and/or animals (American Psychiatric Association, 2013). Given the build-up of cluttering, households of individuals with hoarding disorder (HD) are, in many instances, considered a public health threat. These environmental conditions could lead to infestation and proliferation of insects and rodents, favouring disease transmission (da Cunha et al., 2020; Lockwood, 2018). In animal hoarding situations, improper animal care, such as lack of food and water and small, crowded and unsanitary spaces for animal maintenance are reported (Patronek, 1999), such as in Figure 1.

Leptospira spp. is a zoonotic bacteria that can infect virtually any mammalian species, with a broad spectrum of clinical and subclinical disease (Mohammed et al., 2011). Environmental components can influence leptospirosis epidemiology, such as the occurrence of floods, rainfall indices and the presence of rodents (Naing et al., 2019; Ricardo et al., 2020). Furthermore, rodents have been implicated in the maintenance and spread of leptospirosis in urban centres (de Faria et al., 2008); however, dogs may also play a role in the disease epidemiology (Adler, 2010; Martins et al., 2012). In addition, dogs in close contact with humans, especially in populations with high exposure to risk factors, could play a role as sentinels for environmental contamination and disease surveillance (Bowser et al., 2018).

The potential transmission and spread of zoonotic pathogens have been a public health concern in animal hoarding situations since it may pose a risk to the surrounding community and the individuals with animal HD themselves. Accordingly, this study aimed to assess the seroprevalence of anti-*Leptospira* spp. antibodies in individuals with animal HD and their dogs and associated risk factors in a major city of Southern Brazil.

2 | MATERIAL AND METHODS

2.1 | Sampling and data collection

The study was performed in Curitiba (25°25′47″S and 49°16′19″W), capital of Paraná State, Southern Brazil, based on a previous study that identified 65 households with animal hoarding cases in Curitiba, totalizing 724 dogs (Cunha et al., 2017). A simple random sample calculation designed with 95% confidence level and 5% accuracy was performed. A total of 251 dogs was the minimum sample number established, with sampling as many dogs as possible within each household by convenience. House-to-house dog sampling was carried out in 2017, and due to legal issues, people samplings occurred in 2019. For statistical purposes, all people and dogs living in the household were considered individuals with animal HD and hoarded dogs.

2.2 | Epidemiological data

Epidemiological data were obtained from an objective questionnaire on the environment observation, exposure of individuals with animal HD and their dogs to *Leptospira* spp., as well as their perception about the infection. Concerning environment conditions, variables regarding cat hoarding, object hoarding, presence of rat faeces on the floor and presence of remains of food and trash in the yard were included. Regarding disease perception and exposure of individuals with animal HD, variables included reports of floods, presence of rats in the household and previous knowledge about leptospirosis. Additionally, declaration of a previous diagnosis of leptospirosis and reporting of knowing someone who had leptospirosis were investigated. Regarding dog exposure, variables included living space (inside the home, backyard or both) and feeding habits (food bowls or directly on the floor).

2.3 | Serological diagnosis

Serum samples were screened for *Leptospira* spp. antibodies by a microscopic agglutination test (MAT), as previously described (Fornazari et al., 2012). A collection of 30 serovars was used (described in Table 1). The dilution of 1:100 was used as the cut-off.

2.4 | Statistical analysis

The epidemiological data and frequencies were stratified in three dependent variables to develop the statistical analysis: (1) households

fully assessed (cases in which individuals with animal HD and dogs were sampled), (2) dogs sampled and (3) individuals with animal HD sampled. For each dependent variable, independent variables were selected from an epidemiological questionnaire to evaluate association with *Leptospira* spp. seropositivity.

All variables were evaluated using descriptive and bivariate analyses with frequencies (simple and cross-tables), estimation of odds ratios (ORs), with confidence intervals of 95%, and chi-square test (significance level = 0.05) provided by commercial statistical software (SPSS for Windows, version 16.0, SPSS Incorporated, Chicago, IL, USA).

3 | RESULTS

Although all 65 previously identified households with cases of individuals with animal HD of dogs in Curitiba were visited, dog sampling was allowed in 21 households, totalising 264 dog samples (out of 550 dogs). Regarding human sampling, 11 households were fully assessed, and blood samples were collected from 19 people.

Overall, no human samples were seropositive to anti-*Leptospira* spp. antibodies; however, 16/264 (6.1%; CI 95% 3.3–9.6%) dogs from 11/21 (52.38%) households were seropositive. The proportion of seropositive dogs per case ranged from 5.56% to 20.0%, with titres from 100 to 400. The most frequent serovar was Copenhageni (10/16; 62.5%) (Table 1). Two epidemiological variables were associated with seropositivity: the presence of cats in the household ($p = 0.004$) and reports of flood occurrence ($p = 0.031$) (Table 2).

4 | DISCUSSION

A few studies have previously investigated the occurrence of *Leptospira* spp. in humans and dogs from Southern Brazil (Chapola et al., 2005; Fonzar et al., 2012; Jorge et al., 2017; Polo et al., 2019), and one study focused on studying simultaneously animal owners and their dogs (do Nascimento Benitez et al., 2021). Despite that, to the authors' knowledge, this is the first investigation of *Leptospira* spp. seroprevalence in individuals with animal HD and their dogs.

Although samples from individuals with animal HD analysed were seronegative, the seroprevalence of *Leptospira* spp. in the general human population have been reported ranging from 1.84% to 11% in the same Brazilian region (Chapola et al., 2005; do Nascimento Benitez et al., 2021; Fonzar et al., 2012). Endemic in Brazil, leptospirosis outbreaks have been reported in several regions, with an annual average of 3888 cases and 9.48% fatality, according to the country's surveillance system (Schneider et al., 2015). In addition, Curitiba is one of the cities with the highest mortality rates for human leptospirosis nationwide (Morikawa et al., 2015). The low number of individuals with animal HD who agreed to be sampled may have contributed to the negative results.

Surprisingly, the frequency of anti-*Leptospira* spp. antibodies in the dogs (6.06%) was lower than frequencies found in other studies performed in Southern Brazil, ranging from 9.3% to 23.1% (Fonzar et al., 2012; do Nascimento Benitez et al., 2021; de Paula Dreer et al., 2013; Martins et al., 2013; Pinto-Ferreira et al., 2019). The low occurrence of anti-*Leptospira* spp. antibodies in the dogs may be explained by several factors, such as the low presence of reservoirs in the surveyed region. Furthermore, other factors influence the epidemiology of leptospirosis beyond the presence of rodents and unsanitary environmental conditions, including temperature at the period of sampling, differences in the topography of analysed households and rainfall levels (Azócar-Aedo, 2016; Naing et al., 2019; Ricardo et al., 2020).

Interestingly, another study in the same population of individuals with animal HD and their dogs sampled herein shown a low occurrence of *Toxoplasma gondii* antibodies, despite the observed unsanitary conditions (da Cunha et al., 2020). The social isolation and reclusive nature of many individuals with HD and, consequently, their animals (Nathanson, 2009) may decrease the exposure to both pathogens.

The titres found in dogs may also indicate recent contact with the agent or residual titre from a previous infection (Morikawa et al., 2015). Furthermore, animals may keep low titres when in constant contact with the agent; thus, each case should be individually evaluated considering all related variables. The vaccinal status of the dogs was investigated in the questionnaire; only one (6.25%) seropositive dog had been vaccinated against *Leptospira* spp. strains, at least once. Since this dog's titre was 100 for the Pomona strain, this result could represent a vaccinal response.

The serovar Copenhageni was the most prevalent, corroborating with previous reports in other Brazilian studies (Caldart et al., 2015; Lavinsky et al., 2012). Norway rats (*Rattus norvegicus*) have been described as the primary reservoir for serovar Copenhageni (de Faria et al., 2008). Besides, most individuals with animal HD reported the presence of rats (57.9%), indicating their potential role in the maintenance of this serovar in the studied environments. Despite that, no significant association was found between the seropositivity for *Leptospira* spp. in dogs and investigated variables associated with the presence of rats in the studied households.

IMPACTS

- Household of individuals with hoarding disorder generally involves unhealthy environmental conditions, which can generate several public health concerns, such as potential transmission and spread of zoonotic pathogens to individuals with hoarding disorder, their pets, and the surrounding community.
- Although this is the first report of *Leptospira* spp. serological status in individuals with animal hoarding disorder and their dogs, flooding remains a high associated risk factor for the disease.
- The One Health approach is essential in understanding the epidemiology of infectious diseases, such as leptospirosis.

FIGURE 1 Unsanitary conditions in a household from an individual with animal hoarding disorder

The frequency of dog *Leptospira* spp. seropositivity herein was significantly higher for animals living in households with a reported history of flooding ($p = 0.031$). Such association corroborates previous meta-analyses studies focusing on dogs (Azócar-Aedo, 2016; Ricardo et al., 2020) and human infections (Naing et al., 2019), where flooding is the major risk factor for the spread of the disease.

The presence of cats in the households was significantly associated with the dog's seropositivity ($p = 0.004$). This association may be explained by the worst sanitary conditions associated with concomitant cat and dog hoarding in the same household, possibly increasing their exposure to reservoirs. However, cat body odours have a repelling action of rats (Adduci et al., 2021), contradicting the previous assumption. Further studies are needed to investigate this association.

The results herein indicated that hoarded dogs probably had contact with *Leptospira* spp. at some point in their life. Studies have shown that canine exposure to leptospirosis positively correlates with human infection, playing an important role as sentinels (Jorge et al., 2017; Meny et al., 2019). Although no human seropositivity was found herein, co-habitation with seropositive dogs suggests the presence of the pathogen in approximately half of the households (11/21; 52.38%). In addition, since dogs have been considered potential sentinels in endemic areas (Halliday, 2010), public health programs must become

TABLE 1 Anti-*Leptospira* spp. serology results by microscopic agglutination test (MAT) in dogs from households of individuals with animal hoarding disorder, Curitiba, Paraná, Brazil

	n	%	
Serovars[a]	**16/264**	**6.1**	**Titres (*n*)**
Copenhageni	10/16	62.5	100 (7), 200 (1), 400 (2)
Pyrogenes	2/16	12.5	100 (1), 200 (1)
Pomona	1/16	6.25	100 (1)
Co-infection			
Copenhageni e Pyrogenes	1/16	6.25	100, 100
Copenhageni e Australis	1/16	6.25	100, 100
Pyrogenes e Australis	1/16	6.25	400, 100

[a]Thirty serovars were tested: Australis, Bratislava, Autumnalis, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorraghiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc, Guaricura, Hardjo-prajtino, Hardjo-minis, Hardjo-CTG, Hardjo-bovis and Nupezo-1.

aware of the risk of leptospirosis cases in households of individuals with HD and nearby communities.

The differences in frequencies of *Leptospira* spp. infection between individuals with animal HD and their dogs highlights the importance of a One Health approach for assessing the complex epidemiology of leptospirosis, particularly in vulnerable populations (Polo et al., 2019). The lack of more detailed environmental analyses, such as active investigations for the presence of rodents and the analysis of soil and water in the households, are limitations of this study.

5 | CONCLUSION

In conclusion, despite low sanitary conditions, no individuals with animal HD were seropositive to anti-*Leptospira* spp. antibodies and the seroprevalence in their dogs is among the lowest compared to studies in Southern Brazil. The occurrence of floods in the households was associated with seropositivity, and surprisingly, the presence of cats as well. Serological surveys of dogs in hoarding situations may help

TABLE 2 Bivariate analysis of the epidemiological variables and seropositivity of *Leptospira* spp. antibodies in dogs of individuals with animal hoarding disorder, Curitiba, Paraná, Brazil

Dogs (*N* = 264)		Positive *n* (%)	Negative *n* (%)	Total	OR	95% IC	*p* Value
Sex	Female	12 (7.7)	143 (92.3)	155	2.20	0.69–7.02	0.134
	Male	4 (3.7)	105 (96.3)	109			
Living space	Inside home	0 (0.0)	4 (100.0)	4	–	–	–
	Backyard	15 (6.6)	213 (93.4)	228	2.18	0.28–17.11	0.457
	Both	1 (3.1)	31 (96.9)	32			
Feeding habits	Food bowls	9 (5.5)	155 (94.5)	164	0.77	0.28–2.14	0.401
	On the floor	7 (7.0)	93 (93.0)	100			
Knowledge about Leptospirosis	Yes	13 (5.4)	226 (94.6)	239	0.63	0.08–5.28	0.506
	No	1 (8.3)	11 (91.7)	12			
Presence of cats	Yes	2 (1.7)	119 (98.3)	121	0.15	0.03–0.69	**0.004***
	No	14 (9.8)	129 (90.2)	143			
Object hoarding	Yes	7 (8.2)	78 (91.8)	85	1.69	0.61–4.72	0.225
	No	9 (5.0)	170 (95.0)	179			
Declared history of flooding	Yes	9 (9.6)	85 (90.4)	94	3.38	1.07–10.11	**0.031***
	No	5 (3.1)	155 (96.9)	160			
Presence of food remains	Yes	8 (8.6)	85 (91.4)	93	1.81	0.66–5.00	0.185
	No	8 (4.9)	154 (95.1)	162			
Reported of presence of rats	Yes	10 (5.7)	165 (94.3)	175	1.09	0.33–3.59	0.576
	No	4 (5.3)	72 (94.7)	76			
Presence of rat faces	Yes	7 (5.4)	123 (94.6)	130	0.93	0.31–2.72	0.553
	No	7 (5.8)	114 (94.2)	121			
Trash in the yard	Yes	12 (7.4)	151 (92.6)	163	1.93	0.60–6.15	0.197
	No	4 (4.0)	97 (96.0)	101			

OR, odds ratio.
* Statistical significant value (P<0,05).

develop policies and public health campaigns to prevent human cases of leptospirosis.

ACKNOWLEDGEMENT

The authors are also grateful to the professionals of the Animal Protection Service of Curitiba City. This study was funded by the Araucária Foundation of Paraná (Protocol Number 41,769) and Dr. Cunha fellowship granted by the Coordination for the Improvement of Higher Education Personnel of Brazil (CAPES).

CONFLICT OF INTEREST

The authors report no conflict of interest and have agreed on the manuscript submission.

ETHICS STATEMENT

All stages of this study were approved by the National Human Ethics Research Committee (protocol number 3166749/2019) and the Animal Use Ethics Committee (protocol number 077/2015), both through the Federal University of Paraná. The authors confirm that the ethical polices of the journal have been adhered.

AUTHOR CONTRIBUTIONS

GRC: conceptualisation, data curation, methodology, project administration, supervision, validation, visualisation, writing – original draft preparation, writing – review and editing; **MP**: conceptualisation, data curation, Investigation, methodology, project administration, validation, visualisation, writing – original draft preparation, writing – review and editing; **CMM**: conceptualisation, data curation, formal analysis, methodology, validation, writing - original draft preparation, writing – review and editing; **SMR**: data curation, writing – original draft preparation, writing – review and editing; **ACY**: investigation, writing – original draft preparation, writing – review and editing; **ECS**: investigation, writing – original draft preparation, writing – review and editing; **APS**: conceptualisation, funding acquisition, resources, writing – review and editing; **VMM**: conceptualisation, funding acquisition, writing – review and editing; **HL**: funding acquisition, investigation, resources, writing – review and editing; **AWB**: conceptualisation, funding acquisition, methodology, project administration, resources, supervision, writing – review and editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.704

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How to cite this article: Cunha, G. R. d., Pellizzaro, M., Martins, C. M., Rocha, S. M., Yamakawa, A. C., da Silva, E. C., dos Santos, A. P., Morikawa, V. M., Langoni, H., & Biondo, A. W. (2022). Serological survey of anti-*Leptospira* spp. antibodies in individuals with animal hoarding disorder and their dogs in a major city of Southern Brazil. *Veterinary Medicine and Science*, *8*, 530–536. https://doi.org/10.1002/vms3.704