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Prevalence and risk factors for human leptospirosis in a rural district of Pelotas, a city in southernmost Brazil

Prevalência e fatores de risco para leptospirose humana em um distrito rural de Pelotas, município do extremo sul do Brasil

Prevalencia y factores de riesgo para la leptospirosis humana en un distrito rural de Pelotas, una ciudad en el extremo sur de Brasil

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Abstract

Leptospirosis is one of the most widespread zoonosis in the world. In humans, the disease is responsible for more than a million cases annually. The purpose of this study was to assess human seroprevalence to leptospirosis in a rural community of southernmost Brazil. Furthermore, we assess possible epidemiological cues associated with seroprevalence. A total of 216 individuals were enrolled in the study. With an overall seroprevalence of 21.3% (n=46), to at least one of the 12 antigens tested. Seroreactivity was associated with 13 of the investigated cues, including those associated with the individuals, their behavior, environment, domestic animals, and synanthropic rodents. While seroprevalence was not particularly high when compared to other studies in similar populations, some of the risk factors associated with the disease are easy to circumvent, and indications for policymakers and future studies are made within.

Keywords: *Leptospira*; MAT; Serology; Zoonosis; Neglected disease.

Resumo

A leptospirose é uma das zoonoses mais difundidas no mundo. Em humanos, a doença é responsável por mais de um milhão de casos anualmente. O objetivo deste estudo foi avaliar a soroprevalência humana para leptospirose em uma comunidade rural do extremo sul do Brasil. Além disso, avaliamos possíveis pistas epidemiológicas associadas à soroprevalência. Um total de 216 indivíduos foram incluídos no estudo. Com uma soroprevalência geral de 21,3% (n = 46), para pelo menos um dos 12 antígenos testados. A sororeatividade foi associada a 13 dos fatores de risco investigados, incluindo aqueles associados aos indivíduos, seu comportamento, ambiente, animais domésticos e roedores sinantrópicos. Embora a soroprevalência não tenha sido particularmente alta quando comparada a outros estudos em

populações semelhantes, alguns dos fatores de risco associados à doença são fáceis de contornar e são feitas indicações para formuladores de políticas e estudos futuros.

Palavras-chave: *Leptospira*; MAT; Sorologia; Zoonose; Doença negligenciada.

Resumen

La leptospirosis es una de las zoonosis más extendidas en el mundo. En los seres humanos, la enfermedad es responsable de más de un millón de casos al año. El propósito de este estudio fue evaluar la seroprevalencia humana a la leptospirosis en una comunidad rural del extremo sur de Brasil. Además, evaluamos posibles señales epidemiológicas asociadas con la seroprevalencia. Se inscribieron en el estudio un total de 216 personas. Con una seroprevalencia global del 21,3% (n = 46), para al menos uno de los 12 antígenos analizados. La seropositividad se asoció con 13 de las señales investigadas, incluidas las asociadas con los individuos, su comportamiento, medio ambiente, animales domésticos y roedores sinantrópicos. Si bien la seroprevalencia no fue particularmente alta en comparación con otros estudios en poblaciones similares, algunos de los factores de riesgo asociados con la enfermedad son fáciles de eludir, y las indicaciones para los legisladores y los estudios futuros se realizan en el mismo.

Palabras clave: *Leptospira*; MAT; Serología; Zoonosis; Enfermedad desatendida.

1. Introduction

Leptospirosis is a zoonotic bacterial disease of global distribution, particularly prevalent in tropical and subtropical climates. The causative agents are pathogenic bacteria of the *Leptospira* genus, with over 300 described serovars (Adler, 2015). Transmission occurs through contact with infected animals, their urine or tissues, or indirectly through contact with water or soil contaminated by these (Hartskeerl et al., 2011).

Leptospirosis is considered an emerging, neglected disease, of public health concern, with more than 870 thousand severe human cases every year, and ~49 thousand deaths annually (Picardeau et al., 2014). In the state of Rio Grande do Sul, the southernmost state in Brazil, leptospirosis is particularly burdensome, with an average of 428 cases notified annually (Schneider et al., 2015). True incidence is unknown however, since under diagnosis and under notification is common, especially in low income and/or rural communities, exactly the population considered to be at most risk, due to the occupational aspect of the disease.

Leptospirosis occurs disproportionately in people who practice certain activities or occupations. People working in mines, slaughterhouses, sewers, and farmers and field workers are at particular risk (Benschop et al., 2009). Farmers bring together a series of occupational risks to leptospirosis, such as exposure to water, to humid soils, and to animals, during their daily activities (Chadsuthi et al., 2017). Besides the more obvious direct infection routes affecting these workers, such as bovine to human (Assenga et al. 2015), wild animals also play a role in rural communities. According to Schneider and co-workers (2015), rural populations in Rio Grande do Sul state are eight times more likely to contract leptospirosis than urban populations, especially those associated with the tobacco and rice production industries.

In this light, the purpose of this study was to assess the prevalence of anti-*Leptospira* spp. antibodies in a rural community of the city of Pelotas, Rio Grande do Sul state, Brazil. Furthermore, we assess possible epidemiological cues associated with seroprevalence.

2. Material and Methods

2.1. Study setting and population

In this work, we carried out a cross-sectional observational study (Thrusfield, 2007) of a quantitative nature (Pereira et al., 2018). Participants of the cross-section study were those living in the rural community of Cerrito Alegre (Third district of the city of Pelotas, RS; 31°32’12”S, 52°21’51”W). To be included in the study, participants had to be residents of the third district, over 18 years old, and agree to participate (signing an informed consent). No exclusion criteria were applied, once the inclusion criteria were met. The study was submitted to, evaluated and approved by the ethics committee of the university's school of medicine (CEP/Faculdade de medicina/UFPel, protocol number 1.352.717). According to the 2010 census, Cerrito Alegre has a population of 3074 residents (IBGE 2010). Sample size was calculated considering a confidence of 95% and an expected seroprevalence of 18.5%, for a required minimum of 216 participants.

2.2. Blood sampling

Blood harvesting was carried out by able professional (either a nurse or a physician), with sterile, disposable material, in collection tubes free of anti-coagulants. After harvesting sera were separated through centrifugation, and stored at -20 °C until use.

2.3. Microscopic agglutination test (MAT)

The MAT was carried out as recommended (WHO, 2003), and previously described. Briefly, *Leptospira* spp. strains were cultured in liquid EMJH (Difco) media, at 29 °C, and subcultured every seven days. To use in the MAT, cultures were standardized at 1-2 x 10^8 cells/mL. Serum samples were initially diluted at 1:12.5 in sterile saline, these were incubated with the antigen at a proportion of 1:1 (for a trial titer of 1:25), for two hours at 29 °C. Over 50% agglutination, when compared to control, was considered positive. Positive sera were then re-tested at serial titers, from 1:25 to 1:3,200. *Leptospira* species, serovar, and strains, used in this study can be seen on Table 1.

2.4. Questionnaire and Statistics

Individuals sampled were asked to answer a questionnaire regarding possible risk factors to leptospirosis. Questions regarding the individuals, their behavior, environment, domestic animals, and synanthropic rodents can be seen on Table 2. All statistics were carried out on the Statistical Package for the Social Sciences (SPSS), version 22.0; or on Epiinfo 7. A two tailed Fischer exact test was used to compare exposure and outcome in 2x2 tables. A power of 95% was used, with *p* values of 0.05 or less considered statistically relevant.

3. Results and Discussion

From October 2015 to July 2016, a total of 216 residents of Cerrito accepted to participate in this study. Blood samples were harvested, and all of them answered the epidemiological questionnaire.

Of the sampled individuals, 83 (38.43%) were men, and 133 (61.57%) were women. Of the 216 samples, 46 (21.29%) were seropositive for at least one antigen, at a trial titer of 1:25. Results regarding seroreactivity can be seen in full on Table 1.

Regarding possible risk factors to seroreactivity, environmental issues, such as flooding of the residence (PR: 5.30 - p<0.05); factors regarding the presence of rodents, such as their access to animal fodder (PR: 3.74 - p<0.01); and behavior issues, such as cleaning the water reservatory tank (PR: 3.74 - p<0.01), were all associated with seroreactivity, among others. Full results regarding risk factors for seroreactivity can be seen on Table 2.

Table 1. Antigen list used for the MAT, and results, with titers.

Serovars[ab]	Results and titers						**Total**	%
	25	50	100	200	400	800		
Australis	2	-	2	-	1	1	**6**	13,0
Ballum	5	2	2	-	-	-	**9**	19,6
Canicola	3	2	3	-	-	-	**8**	17,4
Copenhageni	5	1	1	-	-	-	**7**	15,2
Hardjo	3	1	-	-	-	-	**4**	8,7
Pomona	-	2	-	-	-	-	**2**	4,3
Patoc	6	-	-	-	-	-	**6**	13,0
Pyrogenes	-	1	-	-	-	-	**1**	2,2
Ballum/Copenhageni[c]	-	1	-	-	-	-	**1**	2,2
Australis/Pyrogenes[c]	1	1	-	-	-	-	**2**	4,3
Total	25	11	8	-	1	1	**46**	100

[a]When coagglutination occurred, the serovar reacting at the highest titer was considered.
[b]Serovars assessed that did not react: Autumnalis, Bataviae, Grippotyphosa, and Icterohaemorrhagiae.
[c]Coagglutination at the highest titer. Source: This study.

In a rural district of Pelotas, southernmost Brazil, this study found an overall seroprevalence for human leptospirosis of 21.3% (46 of 216) with titers varying from 25 to 800. Pelotas is unique in its diversity of *Leptospira* species and serovars circulating in its animal and human population (Silva et al., 2008, Silva et al., 2009, Silva et al., 2010, Cunha et al., 2016). Nonetheless, serological assessments in humans are few and far between, and none have been undertaken in rural populations, with most epidemiological information regarding notified cases (Schneider et al., 2015). In this light, the randomized sample of apparently healthy individuals found herein allows for a hereto unseen report on *Leptospira* spp. seroreactivity in these populations.

In our study, Ballum and Canicola were the most frequent reacting serovars (19.6% and 17.4% of all the positive sera, respectively). Curiously, these were the two serovars in the test panel that were replaced with local isolates: Canicola/Kito (Silva et al., 2008) and Ballum/4E (Silva et al., 2010).

Table 2. Epidemiological cues assessed and risk factor description for seroreactivity to anti-*Leptospira* antibodies in a rural district of the city of Pelotas (RS).

Variable	Answer	Total	Reactive	%	P.R.	C.I.[a]
Variables regarding the individuals, their home, and their environment						
Cleaning grease trap	Yes	112	30	26.8	2.0	1.0-3.9**
	No	104	16	15.4		
Cleaning water tank	Yes	119	34	28.6	2.8	1.3-5.8*
	No	97	12	12.4		
Fishing	Yes	79	24	30.4	2.3	1.2-4.4**
	No	137	22	16.0		
House prone to flooding	Yes	7	4	57.1	5.3	1.1-24.6**
	No	209	42	20.1		
House in humid location	Yes	34	24	70.6	17.0	7.1-40.5*
	No	170	21	12.3		
Fenced vegetable garden	Yes	124	21	16.9	0.2	0.09-0.4*
	No	46	23	50		
Variables regarding domestic animals and synanthropic rodents						
Cats inside the house	Yes	68	23	33.8	2.7	1.4-5.4*
	No	148	23	15.5		
Livestock fed with concentrated fodder	Yes	97	10	10.3	0.2	0.1-0.5*
	No	115	36	31.3		
Livestock graze in humid fields	Yes	34	24	70.6	17.0	7.1-40.5*
	No	170	21	12.3		
Livestock graze in Dry fields	Yes	156	20	12.8	0.2	0.09-0.4*
	No	59	26	44.1		
Animal access to fodder storage	Yes	17	11	64.7	8.5	2.9-24.5*
	No	197	35	17.7		
Presence of rodents in	Yes	49	20	40.8	3.7	1.8-7.6*

animal fodder	No	167	26	15.6		
Routine rodent control	Yes	161	43	26.7	6.3	1.8-21.3*
	No	55	3	5.4		

[a] *p* value calculated using a two tailed exact Fischer test: * p<0.01; ** p<0.05.
P.R. = Prevalence Ratio; C.I. = Confidence Interval 95%.
Source: This study.

The use of local isolates in screening panels has been shown to increase the sensitivity of MAT (Faine et al 1999). Furthermore, these serovars are associated with domestic and synanthropic animals (Canicola with dogs and Ballum with rodents)(Bharti et al., 2003), present in most of the participants homes.

Schneider and co-workers (2015) indicate rice and tobacco cultures as possible risk factors for leptospirosis in Rio Grande do Sul state, common activities in the studied region, especially tobacco, which is appropriate for the small agricultural units of Cerrito Alegre. Furthermore, we found that use of animal feed, rodents in the animal fodder, loose control over access to animal fodder (animal access), and frequent rodent control measures were associated with seroreactivity in humans (Table 2). Needless to say, rodents are the most common species' associated with human leptospirosis (Zacarias et al., 2008), and the high Ballum seroreactivity, compared to other serovars, supports synanthrpic rodents as the foremost maintenance hosts in the region.

Contact with water through fishing; cleaning water tanks; and cleaning grease traps, were also associated with seroreactivity. In line with this, the residence being prone to flooding, and the high humidity of fields where the livestock are kept were also associated with seroreactivity (Table 2). All these water related risk factors were expected, as these bacteria are easily carried by water, where they can survive for long periods of time (Caminiti et al., 2011). Nonetheless, orientation towards prevention of the disease seldom regards household chores, such as cleaning water tanks, as important risk factors, and the population of these rural communities usually undertakes these activities without specialized help or appropriate equipment. Policymakers should therefore be advised to emphasize the need of protective equipment whenever handling these materials.

Most of the houses where people were interviewed had vegetable gardens. If these were fenced, or otherwise isolated from animals, a protection from seroreactivity to leptospirosis was observed (Table 2). This is likely due to the manipulation of humid soil, which can be a risk factor if dogs, or other animals have urinated on it, since *Leptospira* spp. can survive in these conditions, especially in gardens where the pH of soil is often corrected

to numbers that favor bacterial survival (pH ~6,2)(Acha et al., 2003, Khairani-Bejo et al., 2014). Likewise, the presence of cats was expected to be a protection factor, since previous studies have described it as such, however, our findings reveal the contrary in the studied population, where housed cats were, in fact, a risk factor (Table 2). Cats are known to suffer from leptospirosis, sometimes with relatively high seroprevalence (25.2%)(Azócar-Aedo et al., 2014), but clean habits usually prevent them from transmitting the disease to humans. Nonetheless, these animals may be carrying rodents into the houses, or exposing their owners in other fashions, yet to be revealed.

General seroprevalence was relatively average, compared to similar studies in similar settings which have found both lower (12.2%)(Escadón-Vargas et al., 2017) and higher (34.8%)(Samsudin et al., 2015) seroprevalence. Nonetheless, measures to control the disease are essential and should be undertaken. Policymakers that work with rural populations should emphasize the use of protective equipment when handling water, soil, and animals.

4. Conclusion

In the conditions of this study, rural populations in the city of Pelotas (residents of the third district) have a general seroprevalence of 21.3% to leptospirosis. Further studies in different settings and sustained surveillance in these populations are also recommended to aid control and reduce leptospirosis cases.

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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