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Research Paper

Frequency of anti-*Leptospira* spp. antibodies in dogs and wild small mammals from rural properties and conservation units in southern Brazil

Juliana Aizawa Porto de Abreu[a], Felipe da Silva Krawczak[b,c], Israel Barbosa Guedes[a], Antonio Francisco de Souza-Filho[a], Gisele Oliveira de Souza[a], Lina de Campos Binder[b], Caroline Sobotyk de Oliveira[d], Jonas Sponchiado[e], Geruza Leal Melo[f], Marcelo Bahia Labruna[b], Marcos Bryan Heinemann[a,*]

[a] *Laboratório de Zoonoses Bacterianas, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brazil*
[b] *Laboratório de Doenças Parasitárias, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brazil*
[c] *Setor de Medicina Veterinária Preventiva, Departamento de Medicina Veterinária, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Brazil*
[d] *Departamento de Medicina Veterinária Preventiva, Centro de Ciências Rurais, Universidade Federal de Santa Maria, Brazil*
[e] *Instituto Federal de Educação, Ciência e Tecnologia Farroupilha, Campus Alegrete, Brazil*
[f] *Programa de Pós-Graduação em Biodiversidade Animal, Departamento de Ecologia e Evolução, Universidade Federal de Santa Maria, Brazil*

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ABSTRACT

Leptospirosis is a disease of worldwide distribution that affects man and several animal species. Domestic and wild animals can behave as reservoirs of the agent. The aim of this study was to evaluate the frequency of anti-*Leptospira* spp. antibodies in dogs and wild small mammals from rural properties and conservation units of three municipalities of Rio Grande do Sul State, Brazil. Sera were collected from 192 domestic dogs and 132 small mammals, namely rodents of the Cricetidae and Caviidae families and marsupials of the Didelphidae family. The study used Microscopic Agglutination Test (MAT) against 23 serovars of *Leptospira* spp. Overall, 9.90% (19/192) dogs, and 1.51% (2/132) small mammals were seropositive. *Leptospira interrogans* serovar Australis was the most frequent serovar in dogs and antibodies against *L. interrogans* serovar Pomona were detected in a dog and a small mammal belonging to the same municipality. We conclude that in the regions studied the frequency of seropositivity in dogs is low, and leptospires do not seem to be circulating in small mammals, yet, further research is necessary to assess the real role that these animals may have for leptospirosis in the studied areas. This is the first serological survey in small mammals, including wild rodents, in the area that encompasses conservation units of great importance to Rio Grande do Sul state.

1. Introduction

Leptospirosis is a worldwide distributed zoonosis present in all continents except Antarctica [1]. However, occurrence of leptospirosis is most common in the tropics, especially in South America and Asia [3,12,20]. The disease is found in almost all mammals and affects a wide range of species including companion animals and humans that become infected by direct contact with urine of the carrier mammals or indirect contact with contaminated soil and water [3]. The infections can arise from environmental exposure to contaminated freshwater environments or watered soils where pathogenic *Leptospira* spp. are able to survive for prolonged periods [38]. Synanthropic rats and dogs are often implicated as sources of leptospirosis and can carry *Leptospira* spp. strains of high pathogenicity to humans. However, other animals can also participate as reservoirs in the epidemiological chain of the disease [2].

Clinical leptospirosis is not always present in dogs that can asymptomatically shed leptospires in the urine, which presents a risk of exposure to humans [25,36]. Even when animals are symptomatic, clinical diagnosis is not conclusive since clinical signs of leptospirosis, although characteristic, are not pathognomonic [8].

Wild mammals may play a key role in the epidemiology of leptospirosis, as some are able to carry the bacteria over long distances, favoring the spread of the agent. *Leptospira* spp. has been isolated from a

* Corresponding author.
E-mail address: marcosbryan@usp.br (M.B. Heinemann).

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variety of small mammals belonging to different families [15,21,22,26,28,40].

Rio Grande do Sul State is concerned for the environmental preservation of its area, possessing several important conservation units. Some of these units are in regions composed of Atlantic rainforest and Pampa biomes. In Brazil, this last biome is exclusive to Rio Grande do Sul, occupying 63% of the state's and 2.07% of the country's territory. It contains its own flora and fauna and great biodiversity, not yet completely described [6].

The role of different animals as hosts is likely regionally specific [2]. Not only anthropogenic activities and environmental conditions can influence *Leptospira* spp. serovars circulating from animals to humans, but there is also influence of the reservoir species present [3]. Considering this, the aim of this study was to evaluate the frequency of anti-*Leptospira* spp. antibodies in dogs and wild small mammals from rural properties and conservation units of three municipalities of Rio Grande do Sul State, Brazil.

2. Materials and methods

2.1. Study area

This study was performed in three municipalities: Barra do Quaraí, Cerro Largo and Derrubadas in the state of Rio Grande do Sul, southern Brazil (Fig. 1). Samples were collected in rural properties and conservation units: Parque Estadual do Espinilho (Barra do Quaraí) and Parque Estadual do Turvo (Derrubadas). Characteristics of these areas confer direct contact between humans and animals such as the anthropic action present in the Pampa biome in Barra do Quaraí, and the tourism importance of the Atlantic rainforest area in Derrubadas. Cerro Largo is a transition area between the two biomes, Pampa and Atlantic rainforest.

For the present study, samples were collected during three field campaigns (July to October 2013 and January 2014), as part of another study on the ecology of ticks and tick-borne disease in Rio Grande do Sul [17]. This study was previously approved by the Chico Mendes Institute for biodiversity (ICMBio Permit No. 38502–1) and the Animal Care and Use Committee of the Faculty of Veterinary Medicine of University of São Paulo, Brazil (protocol 2908/2013).

2.2. Blood sample collection

Dogs over 4 months of age of both sexes, purebredbred or crossbreed, were evaluated and showed no sign of acute disease. Dogs were taken spontaneously by owners and their blood was collected from the cephalic vein. The wild small mammals were captured using a total of 80 live-traps (75 Sherman and five Tomahawk) baited with bacon, banana, apple and peanut butter installed for five consecutive nights during each field campaign. Additionally, two pitfall station traps in Cerro Largo and three in Derrubadas and Barra do Quaraí with five buckets of 42.5 cm diameter and 60 cm height in each station, connected by a plastic fence (of at least 30 m long and 50 cm high) [39] were installed for the same period. Trapped animals were then anaesthetized with ketamine and xylazine and blood samples were collected for serological analysis from all the trapped animals. Later, they were identified according to morphologic characteristics [5,23].

The sera were separated by centrifugation (8000*g*, 10 min). A total of 324 sera were analyzed: 192 of dogs and 132 of small mammals (Table 1). The small mammal species captured were rodents of the Cricetidae and Caviidae families and marsupials of the Didelphidae family.

2.3. Serology

The Microscopic Agglutination Test (MAT) was performed using 23 live antigens. *Leptospira borgpetersenii* serovars: Castellonis, Javanica, Tarassovi, Whitcombi; *Leptospira interrogans* serovars: Australis, Autumnalis, Bataviae, Bratislava, Canicola, Copenhageni, Hardjo (Hardjoprajitno), Hebdomadis, Pomona, Pomona (strain GR6), Pyrogenes, Icterohaemorrhagiae, Sentot; *Leptospira kirschneri* serovars:

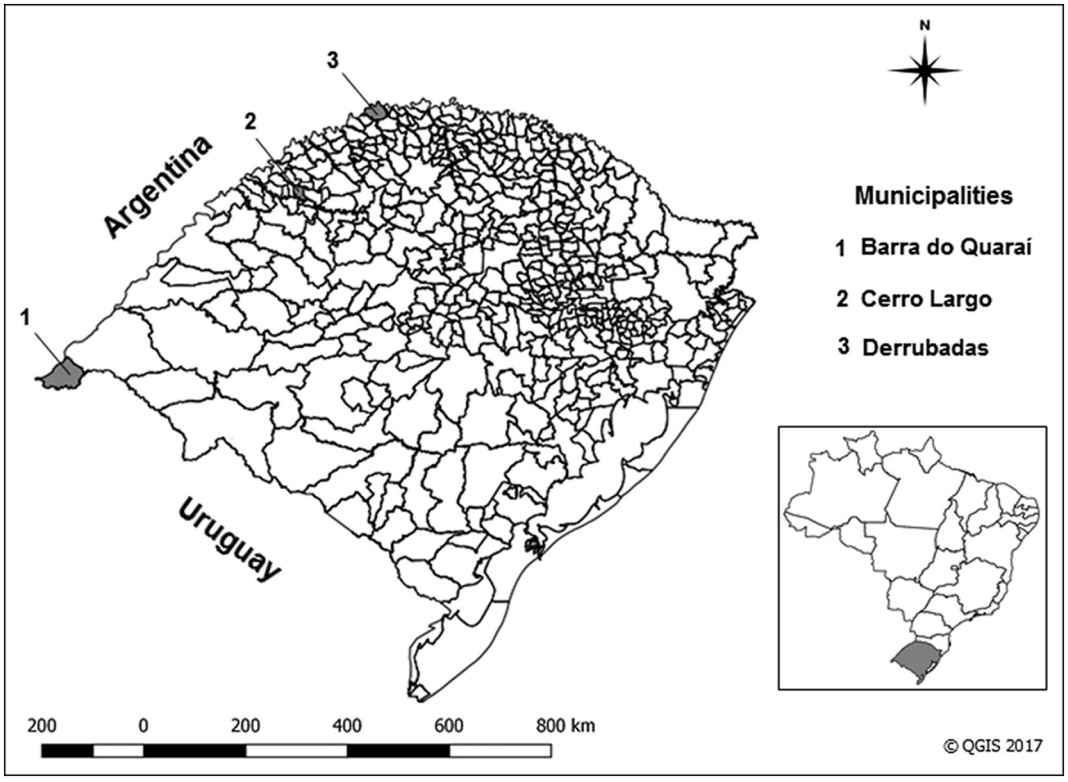

Fig. 1. Location of the three municipalities in the State of Rio Grande do Sul-Brazil, used in this research.

Table 1
Number of analyzed samples of the animals by municipalities used in the study.

Species	Barra do Quaraí	Cerro Largo	Derrubadas	Total
Akodon azarae	16	–	–	16
Akodon montensis	–	17	64	81
Brucepattersonius iheringi	–	–	2	2
Cavia aperea	1	–	–	1
Cryptonanus guahybae	–	–	1	1
Didelphis albiventris	–	3	–	3
Didelphis aurita	–	–	7	7
Euryoryzomys russatus	–	–	1	1
Oligoryzomys nigripes	5	1	3	9
Oxymycterus judex	–	–	6	6
Sooretamys angouya	–	1	3	4
Thaptomys nigrita	–	–	1	1
Canis familiaris	32	127	33	192
Total	54	149	121	324

Butembo, Cynopteri, Grippotyphosa; *Leptospira noguchii* serovar: Panama and *Leptospira santarosai* serovars: Guaricura, Shermani.

The antigens were prepared from reference strains maintained at the Laboratório de Zoonoses Bacterianas of the Universidade de São Paulo; serial passaged in enriched EMJH medium (Difco®), and incubated for seven days at 28 °C. For the screening of seroreactive animals, 1:100 dilution was used. Reactive samples were then examined with increasing dilutions from 1:100 to 1:3200, considering the highest positive dilution to be the titer of the serum [8]. The serum was considered reactive when at least 50% of agglutination occurred at magnification of 100 times under dark-field microscope.

2.4. Statistical analysis

Demographic and serology data were entered in an Excel spreadsheet. The seroprevalence of leptospirosis infection was calculated by dividing the number of seroreactive animals by the total number of animals according to each species. The seroprevalence and their 95% confidence intervals were estimated using the EpiTools (http://epitools.ausvet.com.au).

3. Results

Dog seroprevalence was 9.90% (19/192; CI95% 6.43–13.94), with titers ranging from 100 to 3200, with the highest titer (3200) for the serovar Australis (Table 2). Among the wild small mammals, the seroprevalence in *Akodon montensis* was 2.47% (2/81; CI95% 0.68–8.56), with low titers of serovar Pomona (200) and Grippotyphosa (100). The two reagent animals were rodents from the *Akodon montensis* species and Cricetidae family, both belonging the municipality to Derrubadas. A dog in the municipality to Derrubadas was also positive to Pomona.

4. Discussion

The hosts globally implied with adaptation of serovars Hardjo, Pomona, Icterohaemorrhagiae, Canicola, Grippotyphosa are cattle and sheep, swine, synanthropic rodents, canines, wild rodents and marsupials respectively [3,7,41]. Serovars are adapted to the hosts that favor their persistence in the environment. Those hosts are called reservoir hosts, present subclinical infections and are likely to be the source of infection to other species, through shedding of leptospires in urine. The incidental hosts become infected by direct contact of broken skin or mucous membranes with urine of infected animals or by indirect contact with soil or water containing leptospires and can develop severe acute disease [36]. However, *Leptospira* spp. serovars have different reservoir and incidental hosts depending on the region studied, which can change over time [24].

Considering what is known regarding the epidemiology of leptospirosis in Brazil, in the present study, the dog and wild rodent (*Akodon montensis*) positive to Pomona in Derrubadas municipality are incidental hosts since this serovar is mainly associated with pigs, bovines and also opossums [3,29]. There is less information regarding the other reactive serovars in this study, yet the three most probable serovars in dogs put these in brackets may also be incidental in this species. While Cynopteri uses to bats as maintaining hosts [3], Australis and Butembo are found in wild animals, such as rodents [24,29]. Australis is specifically associated with a reservoir member of the Cricetidae family, *Nectomys squamipes* [29]. In contrast, the rodent reagent to Grippotyphosa was not an incidental host, since this serovar is related to wild mammals as reservoir hosts [24].

Nevertheless, before host–serovar relationships are established it should be noted that the frequency of seropositive animals in this study was low comparing to other serological surveys performed in wild animals and dogs in the country. In Pantanal biome, in Mato Grosso do Sul, a serosurvey performed in pampas deer (*Ozotoceros bezoarticus*) and found 24% (4/17) of seropositivity [19], a posterior study in the dry period showed only 5.9% (3/51) seropositivity in this species [42]. The characteristic of this biome, with flooded areas, may favor the survival and transmission of the pathogen. In the same biome and municipality, Girio et al. [11] found 6,67 (4/60) of seropositivity in different wild animal species. Vieira et al. [43] studied small mammals in Mato Grosso do Sul and found 23.28% (34/146) of positivity. Still in the same biome, but in Mato Grosso, the exposure of wild carnivores (42.7%), domestic dogs (17.5%), and horses (74.1%) was also high [15].

In other regions of the country wild animals and dogs also showed higher occurrence than in the present study, such as dogs studied in the state of Paraíba [4] with 21.4% (61/285) seropositivity, Rio Grande do Norte with 6.8% (25/365) [9], Paraná with 11% per dog-trimester [27], and Pará with 17.15% (47/274) [31]. In two semi-arid regions of Brazil the seropositivity was 5.6% (10/180) in dog and 3.9% (6/152) in wild mammals [34]. Moreover wild canids and wild rats in the municipality of Minas Gerais presented 35% (14/40) and 30.3% (13/43) seropositivity, respectively [13,32].

In Rio Grande do Sul there were also high seropositivity of 27.3% (6/22) in slaughterhouse capybaras [37], 38.75% in bovines (527/1360) [14] and 90.36% (553/612) in equines [35]. Dogs in the rural area of the municipality of Pelotas showed seropositivity (with a cut off 1:50) at even lower frequency than in this study, of 2.66% (13/489) [16]. Previous studies in the urban area had shown higher reactivity of 28.85% [10] and 25.38% [18]. This difference in the presence of anti-*Leptospira* spp. antibodies might be explained by the higher acidity of the soil in rural areas and lower population of reservoirs [16]. In addition, from 13 rats studied in a center of conservation of wild animals in São Paulo [30], all presented seropositivity. To our knowledge this is the first-time wild rodents are surveyed with the MAT for leptospirosis in the studied area, that encompasses conservation areas of great importance to Rio Grande do Sul.

Finally, the antigen collection used for the Microscopic Agglutination Test (MAT) has autochthonous strains of leptospires isolated in Brazil, which improved the diagnosis [33], however most successful isolations occur in frequently studied species such as bovines, dogs and other domestic species. There is a possibility that the small wild mammals studied could host unusual or new serovars other than Grippotyphosa.

We can conclude that *Leptospira* spp. does not seem to be circulating in wild animals in the three regions studied and the frequency of seropositive *Leptospira* spp. in dogs was low.

Animal rights

The study had permit and prior clearance of the Ethics Committee for the Use of Animals (CEUA) of the Faculty of Veterinary Medicine of the University of São Paulo (protocol number 2908/2013) and the Chico Mendes Institute for Biodiversity (ICMBio) (protocol number

Table 2
Titers found for the serovars of *Leptospira* spp. in sera from dogs of the three municipalities of Rio Grande do Sul State.

Dog	City	MAT results											
		Aus	Aut	Bra	But	Can	Cyn	Cop	Ict	Gri	Har	Pan	Pom
1	BQ					100	100						
2	BQ					100	100						
3	BQ					100	200						
4	BQ					100	400						
5	BQ				100								
6	BQ											200	
7	BQ	100											
8	CL	3200											
9	CL	3200											
10	CL										100		
11	CL				400								
12	CL	100		100									
13	CL	400		400				100	100				
14	CL	200											
15	CL	200	100		100								
16	CL	100		100									
17	CL	100		100									
18	CL	400		100			100						
19	DB												200

City: BQ: Barra do Quaraí; CL: Cerro Largo; DB: Derrubadas. Serovar: Aus: Australis; Aut: Autumnalis; Bra: Bratislava; But: Butembo; Can: Canicola; Cyn: Cynopteri; Cop: Copenhageni; Ict: Icterohaemorrhagiae; Gri: Grippotyphosa; Har: Hardjo; Pan: Panama; Pom: Pomona.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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References

[1] B. Adler, A.P. Moctezuma, Leptospira and leptospirosis, Vet. Microbiol. 140 (3–4) (2010) 287–296, https://doi.org/10.1016/j.vetmic.2009.03.012.
[2] V. Barragan, S. Olivas, P. Keim, T. Pearson, Critical knowledge gaps in our understanding of environmental cycling and transmission of *Leptospira* spp, Appl. Environ. Microbiol. 83 (19) (2017) e01190–e01217, https://doi.org/10.1128/AEM.01190-17.
[3] A.R. Bharti, J.E. Nally, J.N. Ricaldi, M.A. Matthias, M.M. Diaz, M.A. Lovett, P.N. Levett, R.H. Gilman, M.R. Willig, E. Gotuzzo, J.M. Vinetz, Peru-United States Leptospirosis Consortium, Leptospirosis: a zoonotic disease of global importance, Lancet Infect. Dis. 3 (12) (2003) 757–771.
[4] C.S.A. Batista, C.J. Alves, S.S. Azevedo, S.A. Vasconcellos, Z.M. Morais, I.J. Clementino, F.A.L. Alves, F.S. Lima, J.O. Araújo Neto, Soroprevalência e fatores de risco para a leptospirose em cães de Campina Grande, Paraíba, Arq. Bras. Med. Vet. Zootec. 57 (suppl.2) (2005) 179–185, https://doi.org/10.1590/S0102-09352005000800008.
[5] C.R. Bonvicino, J.A. Oliveira, P.S. Andrea, Guia dos Roedores do Brasil, com Chaves para Gêneros Baseadas em Caracteres Externos, Centro Pan-Americano de Ferbe Aftosa OPAS/OMS, Rio de Janeiro, 2008http://www.fiocruz.br/ioc/media/livro%20roedores.pdf , Accessed date: 13 October 2017.
[6] Brasil, Ministério do Meio Ambiente Pampa, http://www.mma.gov.br/biomas/pampa, (2017) , Accessed date: 13 June 2017.
[7] W.A. Ellis, Animal leptospirosis, Curr. Top. Microbiol. Immunol. 387 (2015) 99–137, https://doi.org/10.1007/978-3-662-45059-8_6.
[8] S. Faine, B. Adler, C. Bolin, P. Perolat, Leptospira and Leptospirosis, 2nd ed., MedSci, Melbourne, Australia, 1999.
[9] A.R.F. Fernandes, A.G. Fernandes, V.J.A. Araújo, S.S.S. Higino, M.L.C.R. Silva, C.J. Alves, S.S. Azevedo, Soroepidemiologia da leptospirose canina na região metropolitana de Natal, estado do Rio Grande do Norte, Braz. J. Vet. Res. Anim. Sci. 50 (3) (2013) 226–232.
[10] L.R.I. Furtado, M.O. Avila, M.F.B. Fehlberg, M.M. Teixeira, R.L.I. Rosado, L.F.S. Martins, C.S. Brod, Prevalência e avaliação de fatores de risco à leptospirose canina, no Município de Pelotas, RS, Arq. Inst. Biol. 64 (1) (1997) 57–61.
[11] R.J.S. Girio, F.L.G. Pereira, M. Marchiori Filho, L.A. Mathias, R.C.P. Herreira, A.C. Alessi, T.M.S. Girio, Investigation of antibodies to *Leptospira* spp. in wild and feral animals from the region of Nhecolândia, Mato Grosso do Sul, Brazil. Use of the immunohistochemistry technique for the agent detection, Ciência Rural 34 (1) (2004) 165–169, https://doi.org/10.1590/S0103-84782004000100025.
[12] R.E. Goldstein, Canine leptospirosis, Vet. Clin. North Am. Small Anim. Pract. 40 (6) (2010) 1091–1101, https://doi.org/10.1016/j.cvsm.2010.07.008.
[13] D.O. Gomes, G.B. Ramos, V.B. Alves, A.Z. Ciuffa, L.P. Cuccato, T.F. Reis, A.M. Lima, M.C. Gonçalves, G.V. Tolesano, V.S. Rodrigues, M.P. Szabó, Occurrence of anti-Leptospira spp. antibodies in Rhipidomys spp. from a forest fragment of the Brazilian Cerrado, Tropl. Anim. Health Prod. 49 (3) (2017) 555–559, https://doi.org/10.1007/s11250-017-1227-6.
[14] G.P. Herrmann, R.O. Rodrigues, G. Machado, A.P. Lage, E.C. Moreira, R.C. Leite, Soroprevalencia de leptospirose em bovinos nas mesorregiões sudeste e sudoeste do Estado Rio Grande do Sul, Brasil, Ciência Anim. Brasil. 13 (1) (2012) 131–138, https://doi.org/10.5216/cab.v13i1.13190.
[15] R.S.P. Jorge, F. Ferreira, J.S. Ferreira-Neto, S.A. Vasconcellos, E.S. Lima, Z.M. Morais, G.O. Oliveira, Exposure of free-ranging wild carnivores, horses and domestic dogs to Leptospira spp in the northern Pantanal, Brazil, Mem. Inst. Oswaldo Cruz 106 (4) (2011) 441–444.
[16] S.D.D. Jouglard, C.S. Brod, Leptospirose em cães: prevalência e fatores de risco no meio rural do município de Pelotas-RS, Arq. Inst. Biol. 67 (2) (2000) 181–185.
[17] F.S. Krawczak, L.C. Binder, C.S. Oliveira, F.B. Costa, J. Moraes-Filho, T.F. Martins, J. Sponchiado, G.L. Melo, F. Gregori, G. Polo, S.V. Oliveira, M.B. Labruna, Ecology of a tick-borne spotted fever in southern Brazil, Exp. Appl. Acarol. 70 (2) (2016) 219–229, https://doi.org/10.1007/s10493-016-0070-1.
[18] R.R. Machado, C.S. Brod, A.B.P. Chaffe, M.F.B. Fehlberg, L.F.S. Martins, C.B. Ludtke, Leptospirose canina na região sul do Rio Grande do Sul, No ano de, vol. 1998, XIV Congresso Estadual de Medicina Veterinária & III Congresso de Medicina Veterinária do Cone Sul, Gramado, 1999, p. 103.
[19] L.A. Mathias, R.J.S. Girio, J.M.B. Duarte, Serosurvey for antibodies against *Brucella abortus* and *Leptospira interrogans* in pampas deer from Brazil, J. Wildl. Dis. 35 (1) (1999) 112–114.
[20] G. Martins, W. Lilenbaum, The panorama of animal leptospirosis in Rio de Janeiro, Brazil, regarding the seroepidemiology of the infection in tropical regions, BMC Vet. Res. 9 (2013) 237.
[21] M.F. Marvulo, J.C. Silva, P.M. Ferreira, Z.M. Morais, A.M. Moreno, D.S. Doto, R. Paixão, M.R. Baccaro, S.A. Vasconcellos, J.S. Ferreira Neto, Experimental leptospirosis in capybaras (*Hydrochaeris hydrochaeris*) infected with *Leptospira interrogans* serovar Pomona, J. Zoo Wildl. Med. 40 (4) (2009) 726–730.
[22] A. Mayer-Scholl, J.A. Hammerl, S. Schmidt, R.G. Ulrich, M. Pfeffer, D. Woll, H.C. Scholz, A. Thomas, K. Nöckler, *Leptospira* spp. in rodents and shrews in Germany, Int. J. Environ. Res. Public Health 11 (8) (2014) 7562–7574, https://doi.org/10.3390/ijerph110807562.
[23] G.L. Melo, J. Sponchiado, A.F. Machado, N.C. Cáceres, Small-mammal community structure in a South American deciduous Atlantic Forest, Community Ecol. 12 (1) (2011) 58–66.
[24] Z. Milas, Z.S. Majetić, J. Habus, V.M. Perko, V. Staresina, L. Barbic, V. Stevanovic, M. Perharic, B. Ljubic, N. Turk, The occurrence and maintenance of *Leptospira* serovars Autralis and Bratislava in domestic and wild animals in Croatia, Veterinarski Arhiv. 83 (4) (2013) 357–369.
[25] B.A. Miotto, L.Z. Moreno, A.G. Guilloux, G.O. Sousa, A.P. Loureiro, A.M. Moreno,

W. Lilenbaum, S.A. Vasconcellos, M.B. Heinemann, M.K. Hagiwara, Molecular and serological characterization of the first *Leptospira santarosai* strain isolated from a dog, Acta Trop. 162 (2016) 1–4, https://doi.org/10.1016/j.actatropica.2016.06.007.

[26] L.Z. Moreno, F. Miraglia, M.F. Marvulo, J.C. Silva, C.D. Paula, B.L. Costa, Z.M. Morais, J.S. Ferreira Neto, O.A. Dellagostin, R.A. Hartskeerl, S.A. Vasconcellos, A.M. Moreno, Characterization of Leptospira santarosai Serogroup Grippotyphosa Serovar Bananal isolated from Capybara (*Hydrochaeris hydrochaeris*) in Brazil, J. Wildl. Dis. 52 (3) (2016) 688–693, https://doi.org/10.7589/2015-09-245.

[27] V.M. Morikawa, D. Bier, M. Pellizzaro, L.S. Ullmann, I.A.D. Paploski, M. Kikuti, H. Langoni, A.W. Biondo, M.B. Molento, Seroprevalence and seroincidence of Leptospira infection in dogs during a one-year period in an endemic urban area in Southern Brazil, Rev. Soc. Bras. Med. Trop. 48 (1) (2015) 50–55, https://doi.org/10.1590/0037-8682-0213-2014.

[28] A. Obiegala, D. Woll, C. Karnath, C. Silaghi, S. Schex, S. Eßbauer, M. Pfeffer, Prevalence and genotype allocation of pathogenic *Leptospira* species in small mammals from various habitat types in Germany, PLoS Negl. Trop. Dis. 10 (3) (2016) e0004501, , https://doi.org/10.1371/journal.pntd.0004501.

[29] S.V. Oliveira, M.L.N.S. Arsky, E.P. Caldas, Reservatórios animais da leptospirose: uma revisão bibliográfica, Rev. Saúde 39 (1) (2013) 9–20, https://doi.org/10.5902/223658345094.

[30] M.S. Paixão, M.F. Alves-Martin, M.S. Tenório, W.A. Starke-Buzetti, M.L. Alves, D.T. Silva, A.G. Ferreira, M. Floróe Silva, L.O. Sousa, S.B. Lucheis, Serology, isolation, and molecular detection of *Leptospira* spp. from the tissues and blood of rats captured in a wild animal preservation centre in Brazil, Prev. Vet. Med. 115 (1–2) (2014) 69–73, https://doi.org/10.1016/j.prevetmed.2014.03.016.

[31] G.S. Paz, K.S. Rocha, M.S. Lima, E.M. Jorge, J.C.F. Pantoja, C.C.G. Moraes, H. Langoni, Seroprevalence for brucellosis and leptospirosis in dogs from Belém and Castanhal, state of Pará, Brazil, Acta Amazon. 45 (3) (2015) 265–270, https://doi.org/10.1590/1809-4392201403486.

[32] T.C.S. Rodrigues, A.L.Q. Santos, A.M.C. Lima-Ribeiro, F.G. Lemos, F.C. Azevedo, R.C. Arrais, D.O. Gomes, T.C.F. Tavares, Occurrence of antibodies against *Leptospira* spp. in free-ranging wild canids from the Brazilian savana, Pesq. Vet. Bras. 35 (8) (2015), https://doi.org/10.1590/S0100-736X2015000800005.

[33] A.M.C. Sarmento, S.S. Azevedo, Z.M. Morais, G.O. Souza, F.C.S. Oliveira, A.P. Gonçales, F. Miraglia, S.A. Vasconcellos, Use of *Leptospira* spp. strains isolated in Brazil in the microscopic agglutination test applied to diagnosis of leptospirosis in cattle herds in eight brazilian states, Pesq. Vet. Bras. 32 (7) (2012), https://doi.org/10.1590/S0100-736X2012000700003.

[34] L.F. Santos, et al., Seroepidemiological survey on *Leptospira* spp. infection in wild and domestic mammals in two distinct areas of the semi-arid region of northeastern Brazil, Tropl. Anim. Health Prod. 10 (2017) 1–8.

[35] R.F. Santos, G.C.P. Silva, N.A. Assis, L.A. Mathias, Agglutinins to *Leptospira* spp. in equines slaughtered in the southern region of Brazil, Semina 37 (2) (2016) 841–852, https://doi.org/10.5433/1679-0359.2016v37n2p841.

[36] S. Schuller, T. Francey, K. Hartmann, M. Hugonnard, B. Kohn, J.E. Nally, J. Sykes, European consensus statement on leptospirosis in dogs and cats, J. Small Anim. Pract. 56 (3) (2015) 159–179, https://doi.org/10.1111/jsap.12328.

[37] E.F. Silva, N. Seyffert, S.D.D. Jouglard, D.A. Athanazio, O.A. Dellagostin, C.S. Brod, Seroprevalence of leptospiral infection in capybaras (*Hydrochoerus hydrochaeris*) in a slaughterhouse of Rio Grande do Sul, Brazil, Pesq. Vet. Bras. 29 (2) (2009), https://doi.org/10.1590/S0100-736X2009000200016.

[38] R. Thibeaux, S. Geroult, C. Benezech, D. Girault, E. Bierque, C. Goarant, Seeking the environmental source of Leptospirosis reveals durable bacterial viability in river, soils (2017) 1–14, https://doi.org/10.1371/journal.pntd.0005414.

[39] F. Umetsu, L. Naxara, R. Pardini, Evaluating the efficiency of pitfall traps for sampling small mammals in the Neotropics, J. Mammal. 87 (75) (2011).

[40] C. Valbuena-Torrealba, J.E. Péfaur-Vega, Leptospirosis determination in rodents and marsupials from southern Maracaibo lake region, Merida state, Venezuela, Rev. Cient. 25 (3) (2015) 193–199.

[41] S.A. Vasconcellos, O. Barbarini Jr., O. Umehara, Z.M. Morais, A. Cortez, S.R. Pinheiro, F. Ferreira, A.C.M. Fávero, J.S. Ferreira Neto, Leptospirose bovina. Níveis de ocorrência e sorotipos predominantes em rebanhos dos Estados de Minas Gerais, São Paulo, Rio de Janeiro, Paraná, Rio Grande do Sul e Mato Grosso do Sul, Arq. Inst. Biol. 64 (2) (1997) 7–15.

[42] A. Vieira, G.M.S. Rosinha, C.E.O. Oliveira, S.A. Vasconcellos, P.A. Lima-Borges, W.M. Tomás, G.M. Mourão, A.C.R. Lacerda, C.O. Soares, F.R. Araújo, U. Piovezan, C.A. Zucco, A.O. Pellegrin, Survey of *Leptospira* spp. in pampas deer (*Ozotoceros bezoarticus*) in the Pantanal wetlands of the state of Mato Grosso do Sul, Brazil by serology and polymerase chain reaction, Mem. Inst. Oswaldo Cruz 106 (6) (2011) 763–768.

[43] A.S. Vieira, et al., Identificação de mamíferos silvestres do Pantanal Sul-Mato-Grossense portadores de *Leptospira* spp, Ciênc. Anim. Bras. 14 (3) (2013) 373–380 (ISSN 1809-6891).