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# *Leptospira* Seroprevalence in Capybaras from a Brazilian Urban Area

**Alexandre Alberto Tonin[1], Felipe da Silva Krawczak[2], Jéssica Carolina Gomes Noll[3], Camila Tochetto[4], Jorge Luiz Rodrigues Martins[3], Manoel Renato Teles Badke[3], Marcelo Bahia Labruna[2] & Aleksandro Schafer Da Silva[5]**

**ABSTRACT**

***Background*:** Leptospirosis remains the most widespread zoonotic disease in the world. As a clinical entity it is strongly associated with regional occupational and environmental exposures. While the exact global disease burden remains unknown, recent estimates by the leptospirosis Burden Epidemiology Reference Group (LERG) at the World Health Organization have set the number of human cases of severe leptospirosis to over 500,000 per year. This number almost certainly represents an under-representation due to poor surveillance and difficult diagnosis. *Leptospira* spp. belong to the order Spirochaetales, family Leptospiraceae, composed by 13 pathogenic *Leptospira* species with more than 260 serovars. Wildlife species are commonly considered to be important epidemiological carriers, mainly because of their frequent reactivity to *Leptospira* serovars native to their habitat. Capybara (*Hydrochaeris hydrochaeris*), a known leptospirosis host, is a widespread species in South America. However, reports regarding the importance of this animal in the epidemiology of leptospirosis are rare. Therefore, the objective of this study was to report the results of leptospirosis serological survey of capybaras from a residential park area in southeastern Brazil.

***Materials, Methods & Results*:** A total of 172 capybaras were sampled at Itú Municipality, state of São Paulo, southeastern Brazil, from December 2012 to May 2013. Sera samples were examined for *Leptospira* antibodies by the microscopic agglutination test (MAT), using live antigens grown in liquid medium (EMJH). A complete panel of 7 serogroups (including 10 reference serovars) was used as antigens: serogroup Sejroe (serovars Hardjo and Wolffi), serogroup Grippotyphosa (serovar Grippotyphosa), serogroup Canicola (serovar Canicola), serogroup Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni), serogroup Australis (serovars Australis and Bratislava), serogroup Pomona (serovar Pomona), and serogroup Autumnalis (serovar Butembo). As a results, 46 (26.75%) were serologically positive: 29 (63.05%) for serogroup Sejroe, 7 (15.22%) for serogroup Icterohaemorrhagiae; 9 (19.56%) were seropositive for serogroups Sejroe and Icterohaemorrhagiae; and 1 (2.17%) was positive for serogroups Sejroe and Grippotyphosa.

***Discussion*:** *L. interrogans* sensu stricto is responsible for the most frequent and severe cases of human and animal leptospirosis. Considering the results of our serological survey, it is important to reinforce that in tropical countries, mainly Brazil, India, Thailand, Vietnam, Australia and Barbados, serovars belonging to the Icterohaemorrhagiae serogroup (Icterohaemorrhagiae and Copenhageni) are generally the most prevalent. Therefore, the report of different serogroups in capybaras (as observed in our study) it is an important observation reported. Human leptospirosis is usually due to serovars that are maintained by the animal populations of a region, which spread the bacterium on the environment; thus, it may represent an important additional risk factor for human population. Our study identified a greater serum reactivity to strains that belongs to serogroup Sejroe. In Brazil this serogroup is highly predominant in livestock; thus, our main hypothesis is that some animals were contaminated with Sejroe serovars when they were at their natural habitat and, once in the park, they maintained these serovars through cross transmission. Therefore, based on our results, it was possible to observe a significant prevalence of serovars belonging to serogroup Icterohaemorrhagiae, and there was also a high prevalence of samples positive to serogroup Sejroe. Thus, our serologic survey showed that capybaras living in an urban area could represent a risk factor for leptospirosis for the human population eventually exposed.

**Keywords:** leptospirosis, wild host, *Hydrochaeris hydrochaeris*, urban environment.

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[1]Universidade do Oeste de Santa Catarina (UNOESC), Xanxerê, SC, Brazil. [2]Faculty of Veterinary Medicine, Universidade de São Paulo (USP), São Paulo, SP, Brazil. [3]Laboratório de Diagnóstico de Leptospirose, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil. [4]Laboratório de Patologia Veterinária, UFSM, Santa Maria. [5]Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC. CORRESPONDENCE: A.A. Tonin [tonin.aat@gmail.com - Tel.: +55 (49) 3441-7099]. Curso de Medicina Veterinária - UNOESC, Campus II. Rodovia Rovilho Bortoluzzi 480 Km 3,5. CEP 89820000 Xanxerê, SC, Brazil.

## INTRODUCTION

Leptospirosis has traditionally been described in the medical literature as a treatable zoonotic disease endemic to low-income countries in temperate and tropical regions [5]. As a clinical entity it is strongly associated with regional occupational and environmental exposures [27]. While the exact global disease burden remains unknown, recent estimates by the leptospirosis Burden Epidemiology Reference Group (LERG) at the World Health Organization have set the number of human cases of severe leptospirosis to over 500,000 per year [1]. This number almost certainly represents an under-representation due to poor surveillance and difficult diagnosis [20].

This agent belongs to the order Spirochaetales, family Leptospiraceae [14], composed by 13 pathogenic *Leptospira* species with more than 260 serovars [3]. Several species of water plants contaminated by leptospiras have potential to be sources of most serogroups [24]; however, the primary reservoirs for infection are microtine rodents [2,9,30] and various game species [6,21]. Wildlife species are commonly considered to be important epidemiological carriers, mainly because of their frequent reactivity to *Leptospira* serovars native to their habitat [23].

Capybara (*Hydrochaeris hydrochaeris*) is the largest rodent in the world and a widespread species in South America [13]. However, reports regarding the importance of this animal in the epidemiology of leptospirosis are rare, especially in urban areas. Capybaras could be efficient reservoirs for *Leptospira*, because they live in areas with abundant water, an important environmental factor for disease transmission [19]. Based on these aspects, the goal of this study was to report the results of a leptospirosis serological survey of capybaras from a residential park area in southeastern Brazil.

## MATERIALS AND METHODS

### *Animals and blood sampling*

Capybaras were sampled in a residential park area at Itú Municipality, state of São Paulo, southeastern Brazil. The residential park had an area of 484 ha, which contained four lakes, and ≈400 homes interposed by conserved forest areas. Because of a Brazilian spotted fever case (caused by the bacterium *Rickettsia rickettsii* and transmitted by the capybara tick *Amblyomma cajennense*) in a child of the residential park in 2012, its entire capybara population was legally authorized to be culled with the purpose to reduce environmental tick burdens [22]. For this purpose, from December 2012 to May 2013, a total of 172 capybaras were collected by corrals [26], and before been culled, individual blood samples were collected, the sera separated by centrifugation, and stored frozen at -20ºC until further testing.

### *Microscopic agglutination test (MAT)*

Samples were examined for *Leptospira* antibodies by the microscopic agglutination test (MAT) [8,15], using live antigens grown in liquid medium (EMJH)[1] free of contamination or self-agglutination., and as recommended by the World Health Organization [34]. A complete panel of 7 serogroups (including 10 reference serovars) was used as antigens: serogroup Sejroe (serovars Hardjo and Wolffi), serogroup Grippotyphosa (serovar Grippotyphosa), serogroup Canicola (serovar Canicola), serogroup Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni), serogroup Australis (serovars Australis and Bratislava), serogroup Pomona (serovar Pomona), and serogroup Autumnalis (serovar Butembo). Briefly, live suspensions of leptospires representing the 10 serovars were added to serially diluted serum specimens in 96-well microtiter plates and incubated at room temperature for 2 h. Agglutination was examined using dark-field microscopy at × 100 magnification. Titers were calculated as the highest serum dilution that agglutinated at least 50% of the leptospires for each serovar used [7].

## RESULTS

Our results showed that from the 172 sera samples tested, 46 (26.75%) were serologically positive. The serogroups distribution showed that 29 (63.05%) samples were positive only for serogroup Sejroe, 7 (15.22%) only for serogroup Icterohaemorrhagiae, while 9 (19.56%) samples were seropositive for serogroups Sejroe and Icterohaemorrhagiae and 1 (2.17%) sample was positive for serogroups Sejroe and Grippotyphosa. Recent studies conducted in many tropical countries reinforce the complex epidemiological relationship between human/animal leptospirosis [1], reinforcing the importance of our results.

## DISCUSSION

*L. interrogans* sensu stricto is responsible for the most frequent and severe cases of human and animal leptospirosis [34]. Synanthropic and wild rodents have

been considered important *Leptospira* reservoirs [33]. In Brazil, a serological *Leptospira* survey in capybaras culled in a slaughterhouse was performed [31], showing animals with high titers of agglutinating antibodies against a serovar Bratislava and serovar Australis, serovars that belong to serogroup Australis. Differently from this result, our findings serologically classified the samples in other different serogroups, highlighting the potential of infection of this wild species.

The report of different serogroups in capybaras living in an urban area it is an important observation, since human leptospirosis is usually due to serovars that are maintained by the animal populations of a region, which spread the bacterium on the environment [28,32]. Considering the results of our serological survey, it is important to reinforce that in tropical countries, mainly Brazil, India, Thailand, Vietnam, Australia and Barbados, serovars belonging to the Icterohaemorrhagiae serogroup, for example, Icterohaemorrhagiae and Copenhageni, are generally the most prevalent [4,17]. Thus, it may represent an important risk factor, especially considering that changes in human demography during the last 50 years have raised awareness of the emergence of leptospirosis as an urban health problem [20]. For example, in slum settings, endemic transmission of leptospirosis is largely due to circulation of a single serogroup, *L. interrogans* serogroup Icterohaemorrhagiae [10,16,20,29]. In this context, animals subjected to contaminated water sources living in urban areas may have potential to become hosts and diseases carriers for the general population or even to other domestic animals.

Our study identified a greater serum reactivity to strains that belongs to serogroup Sejroe. In Brazil this serogroup is highly predominant in livestock (especially cattle, sheep and goat) [11,12,18]. Considering our studied area as urban, the main hypothesis for this high Sejroe seroprevalence is the report of epizootiological studies indicating that there is a high degree of adaptation between house mice (*Mus musculus*) and serogroup Sejroe [31]. The transition through ruminant hosts was ruled out because the park area is closed, and on that time, there were only capybaras into the area. Another explanation laid on the hypothesis that some animals were contaminated with Sejroe serovars when they were at their natural habitat and, once in the park, they maintained these serovars through cross transmission. Considering the absence of natural hosts for Sejroe serogroup, this serologic information confirms that capybaras may are good hosts for leptospiras, setting them as important epidemiologic subjects.

## CONCLUSION

Based on our results, it was possible to observe a significant prevalence of serovars belonging to serogroup Icterohaemorrhagiae, which use to cause public health problems, due to the severe presentation for some patient. There was also a high prevalence of samples positive to serogroup Sejroe, which considering the absence of natural host for these serovars, indicate that capybaras are able to maintain these serovars into the environment. Therefore, our serologic survey showed that capybaras living in an urban area could represent a risk factor for leptospirosis for the human population eventually exposed.

MANUFACTURER

[1]Becton-Dickinson Biosciences/DIFCO. Detroit, MI, USA.

***Ethical Approval.*** This work was authorized by the Environment State Secretary of the state of São Paulo (authorization no. 96/2012) and was approved by the Ethical Committee of Animal Use of the Faculty of Veterinary Medicine of the University of São Paulo (protocol No. 3104/2013).

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

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