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MICROBIOLOGY

Anti-*Leptospira* spp. antibody test in noncaptive reptiles from urban and peri-urban reas in Brazil's extreme South

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ABSTRACT: The state of Rio Grande do Sul has a great diversity of reptile species distributed throughout its territory. Due to human actions, such as habitat fragmentation, these animals have been frequently observed in urban and peri-urban environments. This facilitates the spread of pathogens between animals and humans, posing a unique health risk, as many diseases are considered zoonoses. Leptospirosis is among the most common zoonoses in the world and is caused by pathogenic species of bacteria of the genus *Leptospira*. The role of reptiles in the cycle of this disease is yet unknown. However, serological studies have demonstrated positivity for antibodies against *Leptospira* spp. in tortoises and snakes, which may indicate that these animals act in maintaining the pathogen in the environment. This observed the presence of anti-*Leptospira* antibodies in rescued reptiles taken to the Center for Rehabilitation of Wild Fauna and Screening Center for Wild Animals at the Universidade Federal de Pelotas (NURFS-CETAS/UFPEL). Samples were collected from 55 animals (39 *Trachemys dorbigni*; 3 *Philodryas patagoniensis*; 3 *Caiman latirostris*; 3 *Salvator merianae*; 2 *Acanthochelys spixii*; 2 *Phrynops hilarii*; 2 *Hydromedusa tectifera*; 1 *Philodryas aestiva*) from August 2022 to December 2023 and tested for 12 reference serovars. Two animals demonstrated positivity, one for the pathogenic serovar Pyrogenes and the other for the pathogenic serovar Canicola. This result reflected the importance of different species besides mammals as potential reservoirs and responsible for maintaining leptospirosis in the environment.
Key words: chelonian, leptospirosis, lizard, zoonosis.

Pesquisa de anticorpos anti-*Leptospira* spp. em répteis de vida livre de áreas urbanas e periurbanas do extremo sul do Brasil

RESUMO: O Rio Grande do Sul possui uma grande diversidade de espécies de répteis distribuídos por todo o Estado. Devido às ações antrópicas, como a fragmentação de habitats, estes animais vêm sendo observados com frequência no meio urbano e periurbano. Isso facilita a disseminação de patógenos entre animais e seres humanos, sendo um risco a saúde única, visto que muitas doenças são consideradas zoonoses. A leptospirose está entre as zoonoses mais comuns do mundo e é causada pelas espécies patogênicas da bactéria do gênero *Leptospira*. Ainda não se sabe o papel dos répteis no ciclo da doença, porém, estudos sorológicos demonstraram positividade para anticorpos contra *Leptospira* spp. em cágados e serpentes, o que pode indicar que estes animais atuam na manutenção do patógeno no ambiente. O objetivo deste estudo foi observar a presença de anticorpos anti-*Leptospira* em répteis oriundos de resgate e levados ao Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres da Universidade Federal de Pelotas (NURFS-CETAS/UFPEL). As amostras foram coletadas de 55 animais (39 *Trachemys dorbigni*; 3 *Philodryas patagoniensis*; 3 *Caiman latirostris*; 3 *Salvator merianae*; 2 *Acanthochelys spixii*; 2 *Phrynops hilarii*; 2 *Hydromedusa tectifera;* 1 *Philodryas aestiva*), durante o período de agosto de 2022 a dezembro de 2023, e testadas para 12 sorovares de referência. Dois animais demonstraram positividade, um deles para o sorovar patogênico Pyrogenes e outro para o sorovar patogênico Canicola. Este resultado demonstra a importância de outras espécies, além dos mamíferos, como potenciais reservatórios e responsáveis pela manutenção da leptospirose no ambiente.
Palavras-chave: lagarto, leptospirose, quelônio, zoonose.

INTRODUCTION

The state of Rio Grande do Sul, in southern Brazil, is known to harbor 128 described reptile species, viz. one caiman, eight two-headed snakes, 11 chelonians, 21 lizards, and 87 snakes (LEMA, 1994; DI-BERNARDO et al., 2004). Currently, these 128 species correspond to 15% of the 795 species recorded in Brazil and little more than 1% of the 1.1733 reptile species known in the world (COSTA & BÉRNILS, 2018; UETZ et al., 2024).

Wild animals and humans are progressively closer due to habitat destruction caused by human actions for territory expansion or production (WOLFE,

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2005). Thus, pathogens had their dissemination facilitated, favoring the occurrence of emerging and re-emerging diseases (HASSELL et al., 2017).

Leptospirosis is among the most common zoonoses in the world and is caused by pathogenic bacteria of the genus *Leptospira*. This pathogen is present in the renal proximal tubes of animals considered reservoirs of the disease and is excreted in the urine. Thus, the contamination of animals and humans can occur directly, when they have contact with urine, or indirectly when there is contact with contaminated soil and/or water (VERMA et al., 2022).

The main animals considered to be reservoirs are synanthropic rodents such as *Rattus norvegicus*, *Rattus rattus*, and *Mus musculus*. Little is known about the role of herpetofauna in the epidemiology of this disease (VERMA et al., 2022). A study conducted in the state of Bahia with noncaptive *Boa constrictor* found anti-*Leptospira* spp. antibodies, and the first detection of DNA of *Leptospira interrogans* in this species' blood (RODAMILANS et al., 2020). Another study conducted in the United States found *Leptospira* spp. in the kidneys of salamanders, toads, and snakes, thus highlighting non-mammals as reservoirs and their importance in maintaining infections in this geographic niche (VERMA et al., 2022). Furthermore, a study conducted in Brazil by BISCOLA et al. (2011) reported DNA of *Leptospira interrogans* using PCR in kidney and liver samples from captive Brazilian lancehead (*Bothrops moojeni*) and noncaptive Cerrado lancehead (*Bothrops pauloensis*), with an identity of 100% and 93%, respectively.

Considering the scarcity of information on the role of reptiles in the cycle of leptospirosis, as well as the large number of rescued animals in the southern region of the state of Rio Grande do Sul, we detected the presence of anti-*Leptospira* spp. antibodies in different noncaptive reptiles rescued in urban and peri-urban areas of Brazil's extreme south.

MATERIALS AND METHODS

We analyzed 55 samples of reptile blood, including 39 from *Trachemys dorbigni*, three from *Philodryas patagoniensis*, three from *Caiman latirostris*, three from *Salvator merianae*, two from *Acanthochelys spixii*, two from *Phrynops hilarii*, two from *Hydromedusa tectifera,* and one from *Philodryas aestiva*. These animals were received in the Center for Rehabilitation of Wild Fauna (Núcleo de Reabilitação de Fauna Silvestre) and Screening Center for Wild Animals (Centro de Triagem de Animais Silvestres) of the Federal University of Pelotas (NURFS-CETAS/UFPEL) in the municipality of Capão do Leão, state of Rio Grande do Sul, Brazil. Some animals were received due to traumas, mostly due to collisions with vehicles, and others were rescued healthy because they were in areas of urban conflict. The samples were collected on arrival in August 2022 and December 2023.

The collected blood was placed in a tube with heparin. Each collected sample was centrifuged at 4.000 rotations per minute (rpm) for 5 minutes. The obtained plasma was aliquoted in a 1.5-mL microtube and later stored in a freezer at -20 °C.

The samples were processed in the Laboratory of Diagnosis and Research on Leptospirosis (LabLepto) of the Universidade Federal de Santa Maria (UFSM). The plasma samples were tested for the presence of anti-*Leptospira* antibodies with the microscopic agglutination test (MAT) (COLE et al., 1973; GALTON et al., 1965; HÄSSLE et al., 2019), using live antigens cultured in liquid Ellinghausen-McCullough-Johnson-Harris (EMJH) medium free of contamination and self-agglutination, as recommended by the WORLD HEALTH ORGANIZATION (2012), and evaluated in a dark-field microscope. The plasma was screened in a dilution of 1:100, and those that presented 50% or more free cells and agglutination were titrated in two-fold geometric dilutions.

A complete table of ten serogroups (including 12 reference serovars) were used as test antigens, viz. Sejroe (serovars Hardjo [subtype Hardjo-prajitno] and Wolffi), Grippotyphosa (serovar Grippotyphosa), Canicola (serovar Canicola), Icterohaemorrhagiae (serovars Icterohaemorrhagiae and Copenhageni), Australis (serovar Bratislava), Pomona (serovar Pomona), Autumnalis (serovar Butembo), Pyrogenes (serovar Pyrogenes), Ballum (serovar Ballum) and Tarassovi (serovar Tarassovi).

RESULTS AND DISCUSSION

Among the animals received for rehabilitation, the most representative group was that of freshwater turtles (n = 45), followed by snakes (n = 4), caimans (n = 3), and lizards (n = 3). Eight species were included in the study, which may indicate their relative abundance and/or frequency in urban conflicts, making them be rescued and taken to the screening center (VERMA et al., 2022).

Studies conducted in different regions of Brazil reported evidence of infection by *Leptospira*

spp. in reptiles, mostly freshwater turtles (OLIVEIRA et al., 2016; ROCHA et al., 2019; MIRANDA et al., 2020). In the state of Rio Grande do Sul, there are reports of seropositivity from a black-bellied slider (*Trachemys dorbigni*) and a Hilaire's side-necked turtle (*Phrynops hilarii*) captured in lakes in the center of the city of Pelotas (SILVA et al., 2009).

In this research, we obtained samples from reptiles from different cities in southern Rio Grande do Sul, including Pelotas, Capão do Leão, Rio Grande, and Pedro Osório. Thus, our study indicated the prevalence of this agent in animals from part of the state's southern region, and, unlike captive animals in zoos, squares, and parks, they can travel long distances, especially during the reproductive season to mate and lay eggs.

Two samples were positive, i.e., 3.63% of the animals had their serum samples reactive to the pathogenic strains of *Leptospira* spp. (Table 1). One of the positive samples comes from a black and white tegu (*Salvator merianae*) from the city of Pelotas, which had a titration of 100 for the serovar Pomona and 200 for the serovar Pyrogenes. Positivity for more than one serovar may indicate a cross-reaction. Thus, the serovar with the highest titration is considered, which in this sample was Pyrogenes (BOLIN, 1996).

The black and white tegu inhabits open regions and forest edges and is very adaptable to new environments, such as urban areas. Thus, it shares flooded areas with synanthropic animals, including rodents, and may become infected through ingestion and/or contact with contaminated water (VERMA et al., 2022). It is an omnivorous species, i.e., its diet consists of insects, birds, rodents, amphibians, fruits, and leaves (BORGES-MARTINS et al., 1998). Therefore, the habits and predatory activity of this species facilitate the transmission of *Leptospira* spp. between rodents and reptiles (VERMA et al., 2022).

A study conducted in Slovenia found antibodies against the serovars Grippotyphosa, Tarassovi, Australis, Canicola, Copenhageni, and Hardjo in different lizard species except for the black and white tegu (LINDTNER-KNIFIC et al., 2013). Therefore, the presence of anti-*Leptospira* spp. antibodies had not yet been described in this species, and ours is the first record.

The other positive sample was from a Hilaire's side-necked turtle from the city of Capão do Leão, which presented a titration of 100 for serovar Canicola. Freshwater turtles are also omnivores, feeding on leaves, insects, fish, mollusks, and even small mammals, which facilitates the transmission of this agent. Furthermore, they share water bodies with synanthropic rodents, which favors infection through contact and/or ingestion of contaminated water (SOUZA, 2004; VERMA et al., 2022).

In the research of SILVA et al. (2009), the seropositive sample of a Hilaire's side-necked turtle

Table 1 - Reptile species evaluated regarding serology for pathogenic serovars of the *Leptospira* spp. bacteria.

Servoras	*Trachemys dorbigni* (n:39)	*Philodryas patagoniensis* (n:3)	*Caiman latirostris* (n: 3)	*Salvator merianae* (n:3)	*Acanthochelys spixii* (n:2)	*Phrynops hilarii* (n:2)	*Hydromedusa tectifera* (n:2)	*Philodryas aestiva* (n:1)
Hardjo-prajitno	-	-	-	-	-	-	-	-
Wolfii	-	-	-	-	-	-	-	-
Grippotyphosa	-	-	-	-	-	-	-	-
Canicola	-	-	-	-	-	1:100 (n:1)	-	-
Icterohaemorrhagiae	-	-	-	-	-	-	-	-
Bratislava	-	-	-	-	-	-	-	-
Pomona	-	-	-	1:100* (n:1)	-	-	-	-
Butembo	-	-	-	-	-	-	-	-
Copenhageni	-	-	-	-	-	-	-	-
Pyrogenes	-	-	-	1:200* (n:1)	-	-	-	-
Ballum	-	-	-	-	-	-	-	-
Tarassovi	-	-	-	-	-	-	-	-

*Positive samples for the same animal.

was for serogroup Icterohaemorrhagiae (serovar Copenhageni), which is mostly transmitted by rats and is the main cause of severe human leptospirosis in urban areas of Brazil.

Seropositivity for serovar Canicola had already been described in other turtle species but not yet in the Hilaire's side-necked turtle. In Brazil, previous species that were reactive to this serovar were the red-eared slider (*Trachemys scripta elegans*), red-footed tortoise (*Chelonoidis carbonaria*), and yellow-footed tortoise (*Chelonoidis denticulata*), all in captivity (SILVA et al., 2010). In Slovenia, one specimen of a noncaptive European pond turtle (*Emys orbicularis*) also tested positive for the same serovar (LINDTNER-KNIFIC et al., 2013).

In our study, we were unable to use PCR to identify the agent. The material used for this technique must be urine or kidney tissue, where one can find the bacterium and be sure that it is being released into the environment. One limitation of our study was the acquisition of urine samples (because most reptiles lack a bladder) and kidney tissue (because few animals died). This test would be ideal to confirm that the reptiles spread *Leptospira* spp. in the environment. However, the seropositivity suggested that these animals are potential reservoirs, highlighting that non-mammalian animals can also take part in the cycle of this disease.

CONCLUSION

In this study, we reported antibodies against two different pathogenic serovars of *Leptospira* spp., in two reptile species. Moreover, this is the first report of the presence of antibodies in the black and white tegu.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

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

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was approved by the Commission of Ethics in Animal Experimentation of the Universidade Federal de Pelotas (UFPel) under registration number 007519/2023-71.

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