Detection of anti-Leptospira spp. agglutinins in captive South American river turtles, Podocnemis expansa

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ABSTRACT
Leptospirosis is a zoonosis transmitted by contact with infected urine or water contaminated with the agent. Searches for Leptospira spp. in reptiles are scarce although most species have contact with aquatic environments. We evaluated the presence of anti-Leptospira spp. antibodies in Podocnemis expansa housed at the Amazonian Zoobotanical Garden, in Belém, Pará state, Brazil. We analyzed 74 serum samples through the microscopic agglutination test using 31 live antigens from different Leptospira spp. serogroups. Thirty samples (40.5%) were positive against Leptospira spp., with titrations between 100 and 3,200 for one or more serogroups. The Hebdomadis serogroup was the most prevalent, with 26 (87%) out of the 30 positive samples, followed by Djasiman, with two (7%) and Celledoni and Bataviae with one (3%) sample each. The detection of anti-Leptospira spp. agglutinins in P. expansa suggests that the aquatic environment is a transmission route for this pathogen among chelonians.

KEYWORDS: chelonians, microscopic agglutination test, Hebdomadis, Djasiman, Celledoni, Bataviae

Detecção de aglutininas anti-Leptospira spp. em tartarugas-da-Amazônia, Podocnemis expansa de cativeiro

RESUMO
A leptospirose é uma zoonose transmitida pelo contato com urina infectada ou água contaminada com o agente. Estudos de Leptospira spp. em répteis são escassos, apesar da maioria das espécies terem contato com ambientes aquáticos. Avaliamos a presença de anticorpos anti-Leptospira spp. em Podocnemis expansa mantidas no Jardim Zoobotânico da Amazônia, em Belém, Pará, Brasil. Analisamos 74 amostras de soro por meio do teste de aglutinação microscópica usando 31 antígenos vivos de diferentes sorogrupos de Leptospira spp. Trinta amostras (40,5%) foram reagentes contra Leptospira spp., com titulações entre 100 e 3,200 para um ou mais sorogrupos. O sorogrupo Hebdomadis foi o mais prevalente, com 26 (87%) das 30 amostras positivas, seguido por Djasiman, com duas (7%) e Celledoni e Bataviae com uma (3%) amostra cada. A detecção de aglutininas anti-Leptospira spp. em P. expansa sugere que o ambiente aquático é uma via de transmissão para esse patógeno entre quelônicos.

PALAVRAS-CHAVE: quelônicos, teste de aglutinação microscópica, Hebdomadis, Djasiman, Celledoni, Bataviae

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Bacteria of the genus *Leptospira* cause leptospirosis, an anthropozoonosis of great public health concern (Mahajan and Daljeet 2008). This pathogen affects humans and domestic and wild animals via direct or indirect contact with urine of infected animals or contaminated water (Cubas and Baptistotte 2014). Studies on *Leptospira* spp. infection in chelonians are limited and little is known about the relevance of these animals as reservoirs of the pathogen and their risk to public health (Andrews et al. 1965; Glosser et al. 1974; Lindtner-Knific et al. 2013; Oliveira et al. 2016; Fornazari et al. 2018). In Brazil, there are a few studies conducted in zoos that provide information on the sanitary status of chelonians regarding leptospirosis (Esteves et al. 2005; Brasil et al. 2013; Rocha et al. 2019). In the Brazilian Amazon region, one turtle species, *Rhinoclemmys punctularia*, was found to test positive for anti-*Leptospira* spp. agglutinins, also in captive individuals (Rocha et al. 2019).

Serological studies using the microscopic agglutination test are used to evaluate the exposure of animals to pathogens and their susceptibility to infection with different *Leptospira* spp. isolates, and have already been used to test for exposure to *Leptospira* in chelonians (Glosser et al. 1974; Lindtner-Knific et al. 2013). In this study, we aimed at detecting the presence of anti-*Leptospira* spp. antibodies in South American river turtles, *Podocnemis expansa* (Schweigger, 1812) (*Podocnemididae*) housed in captivity in a public aquarium in the northern Brazilian city of Belém.

The study was authorized by the Brazilian federal environmental authority Sistema de Autorização e Informação em Biodiversidade – SISBIO license no. 59785-1. Turtles were sampled at Zoobotanical Park Amazonia (Bosque Rodrigues Alves – Jardim Zoobotânico da Amazônia), located in an urban neighborhood of the city of Belém, in the northern Brazilian state of Pará.

The studied specimens inhabit artificial lakes alongside other chelonian species (*Rhinoclemmys punctularia* and *Podocnemis unifilis*). Individuals of both sexes and different age groups originate from apprehensions of illegally traded wild animals or from reproduction in captivity in the park itself. Water in the lakes is replaced only when the compartments are completely drained for cleaning. On one such occasion, in August 2015, 74 *P. expansa* (23 males, 48 females and three juveniles of undetermined sex) were captured manually for blood sampling. About 1–2 mL of blood were collected by venipuncture into the caudal vertebrae or occipital sinus using 3-mL syringes and tubes without anticoagulant. The injection site was cleaned with 2% chlorhexidine prior to injection. The samples were transported to the Zoonosis and Public Health Laboratory of the Institute of Veterinary Medicine of Universidade Federal do Pará (IMV−UFPA), where they were centrifuged to separate coagulated blood from serum. The serum was placed in 1.5 ml Eppendorf® tubes, identified and stored at -20 °C until analysis.

The samples were analyzed by the microscopic agglutination test (MAT), following Faine et al. (1999), with *Leptospira* antigens. *Leptospira* strains were maintained in Ellinghausen-McCullough-Johnson-Harris broth at 29 °C in the laboratory. The group of *Leptospira* antigens used comprised 31 serovars, with four variants of the Hardjo serovar and the strain NUP-1 isolated from canine urine (Canicola serogroup), for a total of 19 serogroups (Australis, Autumnalis, Ballum, Bataviae, Canicola, Celedoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pamana, Pomona, Pyrogenes, Sejroe, Shermani, Andamana, and Seramanga). Serum samples presenting 50% or more of agglutination compared to the control were considered as positives.

Of the 74 serum samples analyzed, 40.5% (30/74) were positive for *Leptospira* spp., with titrations between 100 to 3,200 for one or more serogroups, while no samples showed coagglutination with two or more serogroups (Table 1). Two samples were positive for the Djasiman serogroup, with titrations between 100 and 200, and only one sample was positive for the Celledoni and Bataviae serogroups, with titrations of 400 and 100, respectively.

Although little is known about how *Leptospira* spp. infection in turtles, or their role as possible reservoirs of bacteria in the epidemiological transmission cycle, some studies have already shown a high prevalence in the detection of anti-*Leptospira* spp. in chelonians in North America, such as *Terrapene carolina carolina* with 91% (29/32) of reactive animals (Andrews et al. 1965), 96% (42/46) of *Sternotherus odoratus* and *Pseudemys scripta elegans* (Glosser et al. 1974), and 93.5% (29/31) of *Emydoidea blandingii* in an urban area (Grimm et al. 2015). In Europe, 13.8% (09/65) of *Testudo graeca*, *Testudo hermanni*, *Emys orbicularis*, and the exotic *Trachemys scripta elegans* from Slovenia were positive for anti-*Leptospira* spp. antibodies (Lindtner-Knific et al. 2013), as were 87.5% (14/16) of introduced *Trachemys scripta* in urban lagoons in Italy (Dezzutto et al. 2017).

In Brazil, reported prevalence of *Leptospira* spp. in chelonians was 27.5% (11/40) for *Trachemys dobignyi* and

| Serogroup   | Frequency (%) | Titration |
|-------------|---------------|-----------|
| Hebdomadis  | 87 (05/26)    | (05/26)   |
|             | (15/26)       | -         | (01/26)   |
| Djasiman    | 7 (01/02)     | (01/02)   | -         | -         |
| Celledoni   | 3             | -         | (01/01)   | -         |
| Bataviae    | 3 (01/01)     | -         | -         | -         |
We provide the first record of the presence of antibodies in *P. expansa* to the Hebdomadis, Djasiman, Celledoni and Bataviae serogroups. Our findings demonstrate that *P. expansa* in captive conditions were exposed to different *Leptospira* spp. serogroups, and that it is important to isolate environmental *Leptospira* and to test wild animals kept in captivity in the Amazon region for exposure to *Leptospira*.

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