ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392201901601

SHORT COMMUNICATION

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 quelônios.

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

CITE AS: Souza Rocha, K.; *et al.* 2020. Detection of anti-*Leptospira* spp. agglutinins in captive South American river turtles, *Podocnemis expansa*. *Acta Amazonica* 50: 115-118.

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, *Rhynoclemmys 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 dorbigny* and

Table 1. Distribution of agglutination reactions to detect anti-*Leptospira* spp. antibodies by the microscopic agglutination test (MAT) based on titrations of the most prevalent detected serogroups in the serum of 30 samples from captive South American river turtles (*Podocnemis expansa*).

Serogroup	Frequency (%)	Titration					
		100	200	400	800	1600	3200
Hebdomadis	87	(05/26)	(05/26)	(15/26)	-	-	(01/26)
Djasiman	7	(01/02)	(01/02)	-	-	-	-
Celledoni	3	-	-	(01/01)	-	-	-
Bataviae	3	(01/01)	-	-	-	-	-

Phrynops hilarii from two urban lakes in Pelotas, in the southern state of Rio Grande do Sul (Silva *et al.* 2009) and 45.5% (30/66) for *Phrynops geoffroanus* from urban streams in Jaboticabal, in the southeastern state of São Paulo (Oliveira *et al.* 2016). In the municipal zoo of Ribeirão Preto, São Paulo, 52 chelonians of four species were tested for reaction to anti-*Leptospira* spp. antibodies, among which 59.1% (26/44) *Trachemys scripta elegans*, 50% (01/02) *Phrynops geoffroanus,* 50% (02/02) *Podocnemis unifilis*, and 75% (03/04) *P. expansa* tested positive (Silva *et al.* 2010).

Rhinoclemmys punctularia turtles kept in the same tanks as the *P. expansa* in our study showed a prevalence of 54.8% (17/31) of animals reactive to *Leptospira* spp. (Rocha *et al*. 2019), a high frequency similar to that found in *P. expansa*. Although the animals were kept in the same environment, and blood collection was performed on the same day, only antigen-antibody reactions for the Celledoni serogroup occurred in both species. This suggests that the diversity of infecting strains and the pathophysiology of *Leptospira* spp. is variable among reptile species. The presence of anti-*Leptospira* spp. antibodies in aquatic turtles has been related to prolonged exposure to *Leptospira* present in water (Andrews *et al.* 1965), contact with captive infected individuals (Grimm *et al*. 2015), to the presence of rodents in turtle farms (Lindtner-Knific *et al.* 2013) or carnivorous habits, such as ingestion of small vertebrates that may have been exposed to bacteria (Glosser *et al.* 1974).

Only four serogroups were detected in *P. expansa* in our study, yet the presence of different serogroups in a single species indicates that they circulate in the habitat and that the host species plays a role in the life cycle of the agent (Oliveira *et al.* 2013). Although the source of infection of the turtles is unknown, the aquatic environment was the likely transmission route, since the tanks in which turtles are kept are not cleaned regularly, which may result in the persistence of bacteria in the water and explain the high titration rate of antibodies (Andrews *et al.* 1965).

The detected serogroups were initially isolated from humans (Kmety and Dikken 1993), and later in domestic and wild animals (Rossetti *et al*. 2005; Betance *et al.* 2017; Rajeev *et al.* 2017; Fornazari *et al.* 2018), indicating that these serovars are not species-specific, making studies of their epidemiology more complicated, as transmission networks can be very complex. Little is known about infectious serotypes, their respective wild reservoirs, and their correlation between urban animals and wildlife (Grimm *et al.* 2015). The extent of the Amazon region, and the complexity of its habitats and biodiversity, can lead to regionally differentiated epidemiological cycles, so that turtles are possibly exposed to different serovars throughout their extensive distribution range (Ferrara *et al*. 2017).

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*.

ACKNOWLEDGMENTS

The authors acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the granting of the scholarship and Jardim Zoobotânico da Amazônia for the support in the capture of turtles and collection of biological samples.

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RECEIVED: 10/05/2019
ACCEPTED: 31/12/2019
ASSOCIATE EDITOR: Rodrigo del Valle