



Presence of anti-*Leptospira* spp. antibodies in captive yellow-spotted river turtles (*Podocnemis unifilis*) in the Eastern Amazon

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ABSTRACT: We describe anti-*Leptospira* spp. agglutinin in yellow-spotted river turtles (*Podocnemis unifilis*) in the Amazon region. Ninety-eight serum samples from individuals housed at the Bosque Rodrigues Alves Zoobotanical Garden of Amazonia, Belém, PA, Brazil, were subject to the microscopic agglutination test (MAT) using 19 different *Leptospira* spp. antigen serogroups. Thirty-four of the 98 samples (35%) were reactive, with titers ranging from 100 to 3200, and eight 8 reactive samples (23.5%) co-agglutinated under two or more serovars. The most common serogroup was *Hebdomadis* (26.9%, 7/26), followed by *Semaranga* (23%, 6/26), *Shermani* (19.2%, 5/26), *Djasiman* (11.5%, 3/26), and *Australis* (7.7%, 2/26); *Bataviae*, *Javanica*, and *Sejroewere* represented by a single sample each (3.9%). The presence of turtles reactive to anti-*Leptospira* spp. antibodies implies exposure to the pathogen.

Key words: Testudines, microscopic agglutination test, *Hebdomadis*, Amazon Region.

Presença de anticorpos anti-*Leptospira* spp. em tracajás (*Podocnemis unifilis*) na Amazônia Oriental

RESUMO: Este trabalho descreve a detecção de aglutininas anti-*Leptospira* spp. em tracajás (*Podocnemis unifilis*) na região Amazônica. Obtivemos 98 amostras de soro, as quais foram submetidas ao teste de aglutinação microscópica (MAT), utilizando 31 antígenos de diferentes sorogrupos de *Leptospira* spp. Das 98 amostras de soro coletadas, 35% (34/98) reagiram apresentando titulações de 100 a 3200; 23,53% dessas amostras (8/34) coaglutinaram na presença de dois ou mais sorovares. O sorogrupo mais comum foi *Hebdomadis* com 26,9% (7/26), seguido de *Semaranga* com 23% (6/26), *Shermani* com 19,2% (5/26), *Djasiman* com 11,5% (3/26), e *Australis* com 7,7% (2/26). *Bataviae*, *Javanica*, e *Sejroe* foram representados com apenas uma amostra (3,9%). A presença de tracajás reagentes a anticorpos contra *Leptospira* spp. implica na exposição ao patógeno.

Palavras-chave: Testudines, teste de aglutinação microscópica, *Hebdomadis*, Região Amazônica.

Leptospirosis, a cosmopolitan anthroozoonosis originating from *Leptospira* spp., exhibits pathogenic or saprophytic characters. Studies involving its epidemiological cycle in reptiles are rare in the literature (FORNAZARI, 2017). But, *Leptospira* spp. has been reported in multiple aquatic environments. Therefore, aquatic species with overlapping distributions may be exposed to this pathogenic agent. One candidate species for

examining potential *Leptospira* spp. infection is the yellow-spotted river turtle (*Podocnemis unifilis*), known as tracajá in the Amazon region (MARVULO & CARVALHO, 2014; EBANI, 2017). Therefore, the present study aimed to detect the occurrence of anti-*Leptospira* spp. antibodies in *P. unifilis* maintained at the Bosque Rodrigues Alves Zoobotanical Garden of Amazonia, Belém, PA, Brazil. Captive specimens were selected for this analysis because wild animals

are typically asymptomatic carriers, which may hinder infection diagnosis and treatment. Nevertheless, few serologic investigations have been performed in captivity including zoos, research centers, and breeding centers (SILVA et al., 2010; EBANI, 2017).

The studied specimens inhabited artificial lakes alongside other turtle species. These lakes communicate with tanks containing other animals such as the spectacled caiman (*Caiman crocodilus*). Water is only replaced when the enclosures are completely drained for cleaning, and the 98 *P. unifilis* examined in the present study were captured manually during these full-drain periods, with the exception of those in one tank that could not be emptied; here, a net was used instead. All *P. unifilis* were healthy, without clinical signs of infection.

Blood samples (1–2 mL) were collected aseptically from dorsal tail veins of physically restrained turtles using 3-mL syringes and 25 × 7 needles, immediately stored in tubes without anticoagulant, and sent to the Laboratory of Zoonoses and Public Health, Institute of Veterinary Medicine, Federal University of Pará (IMV/UFPA), Brazil. Samples were centrifuged to extract the serum, and serum samples were transferred to Eppendorf micro tubes and stored at -20 °C for serological tests.

The presence of anti-*Leptospira* spp. antibodies was assessed using the microscopic agglutination test (MAT) (FAINE, 1999), and been used 31 standard strains. The seeds derived from these strains used in the present research were four months old and grown in culture medium at our laboratory, allowing retrieving 19 serogroups:

Australis, Autumnalis, Ballum, Bataviae, Canicola, Celedoni, Cynopteri, Djasiman, Grippytyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pamana, Pomona, Pyrogenes, Sejroe, Shermani, Andamana, and Seramanga. Samples with $\geq 50\%$ agglutination compared to the control sample were considered reactive and were sent for titration with the cutoff point set at 100, considering this value as the dilution.

Thirty-four of the 98 samples (35%) were reactive, eight of which (23.5%) co-agglutinated under two or more serovars under the same titration. As this result precluded the determination of a predominant serovar and frequency was not calculated for these samples. Among the remaining 26 for which frequency could be calculated, the most common serogroup was Hebdomadis (26.9%, 7/26), followed by Semarang (23%, 6/26), Shermani (19.2%, 5/26), Djasiman (11.5%, 3/26), and Australis (7.7%, 2/26). Bataviae, Javanica, and Sejroe were each represented by a single sample (3.9%). Titers ranged from 100 to 3200 (Table 1).

Agglutinins were detected for eight serogroups, with Hebdomadis being the most frequent. This serogroup was not identified for *P. unifilis* in the Amazon, as this species is typically reported in the northern Amazon region. However, a frequent Hebdomadis serogroup has been described for other wild animal species and cattle (MINEIRO et al., 2007; LENHARO et al., 2012; RODRIGUES et al., 2012). Due to the lack of serologic studies in *P. unifilis*, we compared our findings with that obtained for other turtle species, including *Geochelones* spp., red-eared tortoise, Amazonian tortoise, and water tiger.

Table 1 - Serogroups and titers most frequently detected by the microscopic agglutination test for anti-*Leptospira* spp. antibodies in 26 reactive serum samples of yellow-spotted river turtle specimens collected at the Bosque Rodrigues Alves Zoobotanical Garden in Belém, PA, Brazil.

Serogroups	Titer						Total	(%)
	100	200	400	800	1600	3200		
Australis	–	1	1	–	–	–	2	7.7
Bataviae	–	–	–	–	–	1	1	3.9
Djasiman	1	1	1	–	–	–	3	11.5
Hebdomadis	2	3	2	–	–	–	7	26.9
Javanica	–	–	–	1	–	–	1	3.9
Shermani	1	1	3	–	–	–	5	19.2
Semarang	3	1	1	–	–	1	6	23
Sejroe	1	–	–	–	–	–	1	3.9
Total	8	7	8	1	0	2	26	100

Reactivity to Semarang (serovar Patoc), the second most common serogroup in our study, has been reported in captive *Geochelones* spp. And *Trachemys scripta* (pond slider) (ESTEVEES et al., 2005; SILVA et al., 2010). Despite originating from the non-pathogenic and saprophytic *Leptospira biflexa*, detecting Semarang has important implications for anthrozoosis because its presence may stimulate an immune response and trigger cross-reactions with other pathogenic serovars (MYERS, 1976; PAZ et al., 2015). This might be the reason why Semarang and Bataviae serogroups had the highest titers (3200). Bataviae has also been detected in *Trachemys dorbigni* (D'Orbigny's slider) (SILVA et al., 2009).

Because the water at the enclosures where *P. unifilis* live are freely accessible to other species, it may become a reservoir of *Leptospira* spp. Mammals, in particular, might be sources of infection for *P. unifilis*. Thus, serological tests for the diagnosis of serovars belonging to the serogroups identified in the present study have been conducted in rodents; PAIXÃO et al. (2014) detected samples of rats captured in a wild animal preservation reactive to the serogroups identified for *P. unifilis*, while MESQUITA et al. (2018) detected samples of *Didelphis marsupialis* (common opossum), a common mammal in the region, reactive to the icterohaemorrhagiae and Nupezo serogroups, which are different from the ones detected in this study.

The scarcity of published serogroup research on wild animals inhabiting the study region does not convey an appropriate context for discussing the diversity of serogroups identified in the present study. A better understanding of the serovars present in the study region may allow us to consolidate the serogroups reported here. Data on *P. unifilis* origin and associations with other species prior to arrival at our study site would also greatly benefit future serogroup analyses.

In addition to regular serologic tests on animals to better understand the infection risk, hygienic and sanitary measures are necessary in captive environments. These include regular sanitization of enclosures, removal of synanthropic animals, analysis of water quality, and implementation of a quarantine period before the introduction of a new specimen (PÉREZ-FLORES et al., 2016; ESTEVES-MACHADO et al., 2010; RATAJ et al., 2011; RODRIGUES et al., 2016; DETTUZZO, et al., 2017; EBANI, 2017). Such measures can minimize pathogen incidence in zoos and other facilities where animals are held captive.

In conclusion, this study successfully detected anti-*Leptospira* spp. antibodies in *P. unifilis*, an Amazonian turtle species held in captivity, providing direct evidence that this population has been exposed to the pathogen.

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ETHICS AND BIOSAFETY COMMITTEE

This study was approved by the Brazilian Biodiversity Authorization and Information System - SISBIO (permit no. 59785-1).

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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