

Identification of antibodies to *Leptospira* spp. in the spot-legged turtle (*Rhinoclemmys punctularia*) maintained in captivity

Identificação de anticorpos para *Leptospira* spp. em aperemas (*Rhinoclemmys punctularia*) mantidas em cativeiro

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Abstract

Leptospirosis is a zoonosis of worldwide distribution in which the agent can infect several animal species and accidentally humans. In view of the limited number of studies on anti-*Leptospira* antibodies in wild animal species, especially those living in aquatic environments, we sought in this study to investigate the presence of these antibodies in the spot-legged turtle (*Rhinoclemmys punctularia*) maintained in captivity in the Rodrigues Alves Botanical Garden–Amazon Zoobotanical Park, located in Belém, Pará State, Brazil. Serum samples were collected from 31 turtles, and identification of anti-*Leptospira* antibodies was performed using the microscopic agglutination test, using a collection of 31 live antigens which represent 19 serogroups of *Leptospira*. Among the analyzed samples, 54.83% (17/31) were observed to be reactive, and co-agglutination was detected in a further six samples which were not accounted for in the frequency of serogroups. The most frequently detected serogroups were Tarassovi 72.72% (8/11), Celledoni 18.18% (2/11), and Pomona 9.09% (1/11)], with titers ranging from 200 to 400, being this the first study to report infection of these serogroups in this species of chelonios. The animals were kept in water tanks, which probably favored the transport of the agent and allowed its transmission to the spot-legged turtle. We thus confirmed presence of anti-*Leptospira* antibodies in chelonians maintained in the Rodrigues Alves Botanical Garden.

Key words: Leptospirosis. Microscopic agglutination test. Wild animal.

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Resumo

A leptospirose é uma zoonose de distribuição mundial, na qual o agente pode infectar várias espécies animais e acidentalmente, humanos. Tendo em vista o número limitado de estudos sobre anticorpos anti-*Leptospira* em espécies de animais silvestres, especialmente aqueles que vivem em ambientes aquáticos, buscou-se neste estudo investigar a presença desses anticorpos em aperemas (*Rhinoclemmys punctularia*) mantidas em cativeiro no Jardim Botânico Rodrigues Alves - Parque Zoobotânico da Amazônia, localizado em Belém, estado do Pará, Brasil. Amostras de soro foram coletadas de 31 tartarugas e a identificação de anticorpos anti-*Leptospira* foi realizada pelo teste de aglutinação microscópica, utilizando-se uma coleção de 31 antígenos vivos que representam 19 sorogrupos de *Leptospira*. Entre as amostras analisadas, observou-se que 54,83% (17/31) foram reativas, e detectou-se co-aglutinação em mais seis amostras que não foram contabilizadas na frequência de sorogrupos. Os sorogrupos mais frequentemente detectados foram Tarassovi 72,72% (8/11), Celledoni 18,18% (2/11) e Pomona 9,09% (1/11), com títulos variando de 200 a 400, sendo este o primeiro estudo a relatar a detecção de anticorpos anti-*Leptospira* spp. nesta espécie de quelônio. Os animais eram mantidos em tanques de água, o que provavelmente favoreceu o transporte do agente e permitindo sua transmissão para as aperemas. Assim, confirmou-se a presença de anticorpos anti-*Leptospira* em quelônios mantidos no Jardim Botânico Rodrigues Alves.

Palavras-chave: Leptospirose. Teste de aglutinação microscópica. Animal selvagem.

Rhinoclemmys punctularia (the spot-legged turtle), popularly known as Perema or Aperema, is a semiaquatic chelonian, commonly found in regions of Central and South America (FIGUEIREDO et al., 2010). This species is generally distributed in areas with aquatic systems, such as coastal wetlands, flooded savannahs, primary and secondary forest water bodies, streams, swampy meadows, ponds, and temporary pools (FRETEY et al., 2007; RUEDA-ALMONACID et al., 2007).

Although reptiles are known to be reservoirs of various zoonotic bacteria (EBANI, 2017), the role of poikilothermic animals in the maintenance and dissemination of *Leptospira* is still poorly understood (LINDTNER-KNIFIC et al., 2013). Few studies have examined the true epidemiological status of chelonians in relation to leptospirosis, particularly those maintained in captivity due to a lack of knowledge regarding the sanitary profile of wild animals.

Leptospirosis is an infectious–contagious disease that has a zoonotic character and frequently occurs in tropical regions (ACHA; SZYFRES, 2003). The bacterial agents are species of *Leptospira*, which require hydric conditions to facilitate survival and

transmission, and can infect humans and domestic and wild animals (DEZZUTTO et al., 2017). Furthermore, as an anthroozoonosis, leptospirosis can affect the zookeeper of captive animals (ACHA; SZYFRES, 2003).

In the case of chelonians, the dissemination of *Leptospira* can be catastrophic in populations of wild animals, and may even pose the threat of extinction (GRIMM et al., 2015). Oliveira et al. (2016) detected *Leptospira* spp. in the droppings of *Phrynops geoffroanus* (Geoffroy's side-necked turtle), which led to the conclusion that the turtles harbor and disseminate these bacteria, thereby potentially contaminating the environment.

Although there has been a gradual increase in the number of serological investigations in chelonians, there have to date been no such studies on *R. punctularia*, which makes it difficult to assess the extent of *Leptospira* infection in these turtles. Consequently, in the present study, we aimed to evaluate the serologically reactive frequency of *Leptospira* spp. for *R. punctularia* maintained in the Rodrigues Alves Botanical Garden–Amazon Zoobotanical Park, located in Belém, Pará State, Brazil.

The research reported herein was authorized by the Brazilian Biodiversity Authorization and Information System (SISBIO) no. 59785-1. The study was carried out at the Rodrigues Alves Botanical Garden–Amazon Zoobotanical Park (1°25'51.8''S, 48°27'28.7''W), which covers an area of 15 hectares located in an urban district of Belém. It preserves an important diversity of fauna and flora species of the Amazonian ecosystem and is accordingly considered an important institution with regards to Amazon biodiversity conservation (CARDOSO, 2018). Such characteristics make it an important ecotourism location within the municipality.

Due to the cleaning and disinfection procedures of aquatic tanks in which some species of captive chelonians are maintained, including *R. punctularia*, there was a need for identification and handling of the animals, which eventually favored the collection of blood samples.

After adequate and careful physical restraint, blood samples were collected from 31 *R. punctularia* individuals by puncturing the caudal vertebral vein or occipital sinus using 3-mL syringes and 20 × 5.5 needles. These samples were stored in tubes without anticoagulant and were subsequently sent to the Zoonoses and Public Health Laboratory of the Veterinary Medicine Institute of the Federal University of Pará (VMEI/FUPA), where they were centrifuged at 2000 rpm for 10 minutes for

clot retraction and serum separation. The resulting serum samples were then transferred to marked microtubes and subsequently maintained at -20°C until processed.

Identification of antibodies to *Leptospira* spp. in the serum samples was performed using the microscopic agglutination test, using live antigens representative of 31 serovars belonging to the serogroups Australis, Autumnalis, Ballum, Bataviae, Canicola, Celledoni, Cynopteri, Djasiman, Grippotyphosa, Hebdomadis, icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Sejroe, Shermani, Andamana, and Semarang. The samples showing an antigen–antibody agglutination reaction with a grade of at least 50% from the cut-off point 1:100 dilution at the screening step and the highest dilution at the titration step, were considered as reactive.

Of the 31 samples collected, 54.83% (17/31) were reactive for *Leptospira* spp. with titers ranging from 100 to 400 for one or more of the serogroups. We also detected co-agglutination in six samples that made it impossible to identify the predominant serogroup, and accordingly these sample were not included in the frequency data. Among the reactive samples, the most frequently detected serogroup was Tarassovi 72.72% (8/11), with titers ranging from 200 to 400, followed by the serogroups Celledoni 18.18% (2/11) and Pomona 9.09% (1/11) (Table 1).

Table 1. Distribution of sero-reactive samples in the detection of anti-*Leptospira* antibodies using the microscopic agglutination test according to the titers of the most frequently detected serogroups in spot-legged turtle (*Rhinoclemmys punctularia*) maintained in captivity in the Rodrigues Alves Botanical Garden–Amazon Zoobotanical Park.

Serogroups	Frequency (%)	Titers	
		200	400
Tarassovi	72.72% (8/11)	(2/11)	(6/11)
Celledoni	18.18% (2/11)	(1/11)	(1/11)
Pomona	9.09% (1/11)	(1/11)	-
Total	100% (11/11)		

Although research on the occurrence of *Leptospira* spp. in the species studied is still in the early stages, some studies (ANDREWS et al., 1965; DEZZUTTO et al., 2017; GLOSSER et al., 1974; GRIMM et al., 2015; OLIVEIRA et al., 2016) have indicated that chelonians can serve as possible reservoirs of these agents, showing high prevalence rates similar to those detected of the present study.

Our findings regarding the serogroup Tarassovi, which was most frequently detected in the positive reactions recorded in *R. punctularia*, are consistent with the findings reported by Glosser et al. (1974) for *Pseudemys scripta elegans* inhabiting settling ponds of an untreated sewage disposal system in the United States. Similar reactions have also been reported by Lindtner-Knific et al. (2013) in *Emys orbicularis* and *Trachemys scripta elegans* in Slovenia, and by Dezzutto et al. (2017) in *Trachemys scripta* in three urban lagoons in Italy.

In Brazil, Silva et al. (2009) found that *Trachemys dorbigny* from urban lakes in the municipality of Pelotas, Southern Brazil, were also reactive for the Tarassovi serogroup; however, the largest number of animals were reactive for the Bataviae serogroup, which contrasts with the results of the present study.

Although the Tarassovi serogroup has human origins (KIKTENKO & ANANYIN, 1941), it is one of the most frequently detected serogroups in snakes and chelonians, which, according to Esteves et al. (2005), occurs as a consequence ingesting prey infected by the causal agent.

The serogroup with the second highest number of reactions in the present study was Celledoni and we were unable to find any available literature reports of chelonians infected by this serogroup. Accordingly, the present study is probably the first to detect these agglutinins in *R. punctularia*. The serogroup Pomona had the lowest frequency of serum reactants, which has also been reported by Lindtner-Knific et al. (2013) in *Testudo graeca* from a reptile breeding farm in Slovenia.

When comparing the results of the present study with those of studies previously conducted in Brazil, there are notable discrepancies in the reported infectious serogroups in chelonians, as demonstrated by Oliveira et al. (2016), who found that serovar Copenhageni from the serogroup Icterohaemorrhagiae was more frequently detected in samples collected from the eastern box turtle (*Terrapene carolina carolina*). Similarly, Silva et al. (2010) found that pond turtles of the Emydidae family maintained in the municipal zoo of Ribeirão Preto, São Paulo State, Brazil, harbored Andamana.

Although we are unable to verify the source of infection observed in *R. punctularia*, we suspect that exposure to the agent possibly occurred via the water contained in the tanks they inhabit, which may have been contaminated by bacteria disseminated by natural reservoirs, such as rodents and other infected animals, that are often attracted by the food remains left in the enclosures (LOPES et al., 2005; MACHADO et al., 2010).

In addition, the zoobotanical park contains a large diversity of flora, including species of trees with massive canopies. These trees can substantially reduce the incidence of sunlight filtering down to some enclosures, including the tanks containing chelonians, and this, in association with rainfall and high temperatures and humidity, may favor the spread of the infectious agent (MACHADO et al., 2010).

On the basis of the findings of this study, we can conclude that the spot-legged turtle (*Rhinoclemmys punctularia*) maintained in captivity in the Rodrigues Alves Botanical Garden–Amazon Zoobotanical Park are exposed to different serogroups of *Leptospira* spp. To the best of our knowledge, this is the first study to report the detection of agglutinins of the serogroups Tarassovi, Celledoni, and Pomona in this species of pond turtle.

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Conflict of Interests

The authors declare that there are no conflicts of interest.

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