



Communication

[Comunicação]

Monitoring of *Leptospira* in captive turtles by DNA analysis

[Monitoramento de *Leptospira* em quelônios mantidos em cativeiro pela análise de DNA]

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Zoological and botanical parks play a fundamental role in the conservation of biodiversity, and serve as valuable sources of information (Costa, 2004). However, the spread of pathogens among different confined wild species is highly probable due to population densification in smaller facilities (Cubas, 2008). One of the important zoonotic agents is *Leptospira* spp., a pathogen that can infect different animal species without necessarily causing any clinical symptoms, and that can transform the hosts into potential carriers (Ebani, 2017). Molecular diagnostic techniques can effectively detect this pathogen with high sensitivity and specificity (Cordeiro *et al.*, 2017).

Only a few studies on infectious diseases in wild animals have been reported so far; specifically, the data on infectious diseases in reptiles are very limited. Furthermore, there is limited knowledge about the epidemiology of *Leptospira* spp. infection in these reptiles (Ebani, 2017). Certain aquatic species of turtles may be more exposed to *Leptospira* spp., as these bacteria are well adapted to aquatic environments and can survive for several months in moist soils and fresh water (Plank and Dean, 2000). Thus, the constant contact between these captive-bred species and caretakers/professional zookeepers may represent a risk of bacterial transmission to the people

belonging to this occupational group (Ebani, 2017). The aim of this study was to search for *Leptospira* spp. in blood samples from chelonians kept in captivity at Bosque Rodrigues Alves (Jardim Botânico da Amazônia) and thus contribute new reports.

The aquatic turtles in the present study resided in artificial lagoons inside the Bosque, where the water was not managed regularly. A total of 148 turtles belonging to six different species were included in the study; these consisted of 98 males, 38 females, and 12 individuals of undefined sex due to their very young age, weighing 540 g to 21.92kg, with no clinical signs of infection (Table 1). The authorization for the collection of biological material for this study was granted by the Biodiversity Authorization and Information System (SISBIO) issued based on Normative Instruction n° 03/2014, with the authentication code: 59238645.

Blood samples were collected aseptically after physically restraining the animals carefully to minimize stress. Around 1–2mL of blood were collected in anticoagulant tubes from the dorsal caudal vein or occipital sinus of the animals using 3mL syringes (Figure 1) and stored at -20°C for subsequent analysis.

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Table 1. Categorization of turtles included in this study based on the species, common name, and habitat

Species	Common name	Habitat	Number of animals
<i>Podocnemis expansa</i>	Amazonian-turtle	Aquatic	10
<i>Podocnemis unicifilis</i>	Tracajá	Aquatic	95
<i>Podocnemis sextuberculata</i>	Iaçá/Pitiú	Aquatic	4
<i>Rhinoclemmys punctularia</i>	Perema	Aquatic	30
<i>Chelonoidis denticulatus</i>	Yellow-jabuti/Jabuti-tinga	Terrestrial	1
<i>Chelonoidis carbonarius</i>	Red-jabuti/Jabuti-piranga	Terrestrial	8
Total			148

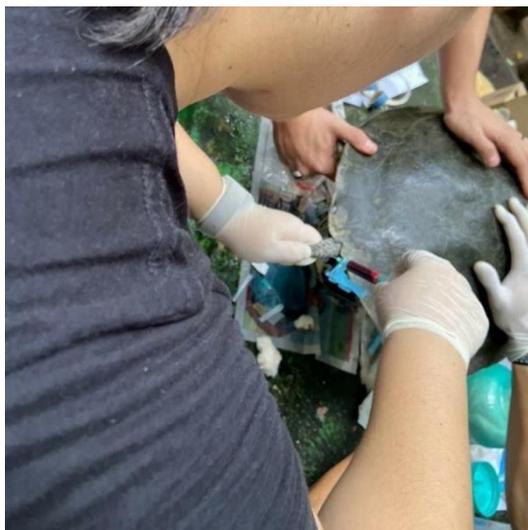


Figure 1. Blood collection from Amazonian-turtles (*Podocnemis expansa*) through puncture of the dorsal caudal vein. Source: Ellen Yasmin Eguchi Mesquita.

DNA was extracted from the blood samples using the ReliaPrep™ Blood gDNA Miniprep System (Promega®, USA) following the manufacturer's protocol. The sequences of the primers specific to *Leptospira* spp. DNA, Lep 1 and Lep 2 as reported by Merien *et al.* (1992), were obtained from GenBank. Further, 5µL of DNA extracted from *Leptospira*, obtained from culturing *Leptospira interrogans* serovar Canicola in EMJH, and 5µL of sterile water were used as a positive and negative control, respectively. Polymerase chain reaction (PCR) was performed to detect a 331bp fragment following the protocol described by Rocha (2016).

The products were run on a 1.5% agarose gel and stained with Safer dye (Kasvi®, Brazil) to visualize the amplified fragments and verify DNA quality and integrity. The samples were visualized under an ultraviolet (UV) transilluminator coupled to a photo documentation system (Gel Doc™ XR+ Imager, Bio-Rad®, USA). Our

experiment showed that all the animals included in this study were negative for *Leptospira* spp. This is the first time a molecular diagnostic technique was used to detect *Leptospira* spp. in blood samples collected from turtles (Figure 2).

Alves Jr (2013) analyzed urine and stomach content samples from 120 free-living Amazonian-turtles (*Podocnemis expansa*) by PCR using the Lep 1 and Lep 2 primers and detected *Leptospira* spp. DNA in at least two stomach content samples. The author also analyzed samples from 480 Amazonian-turtles (*P. expansa*) bred in captivity and detected the bacterial DNA in two urine samples. In contrast to the present study, this author assayed different types of biological samples, which may have aided the detection of the bacteria. Another study by Oliveira (2013) detected *Leptospira* spp. in 11 samples, including eight stomach wash and three cloacal wash samples, from 66 Geoffroy's side-necked turtles (*Phrynops geoffroanus*).

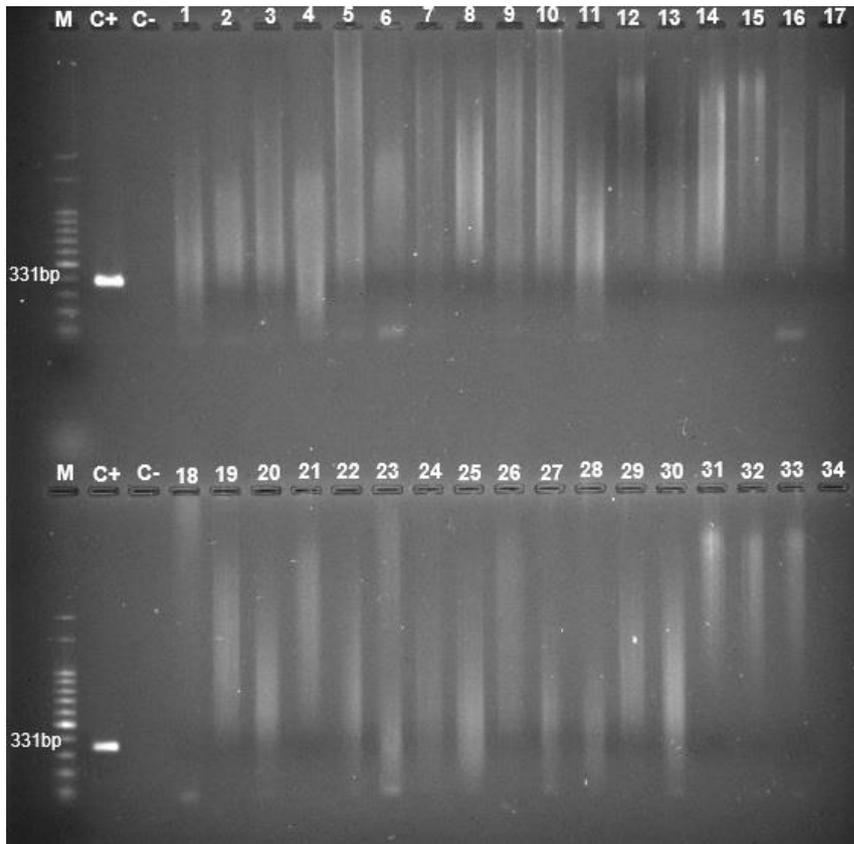


Figure 2. Results of the PCR analysis visualized on 1.5% agarose gel. (M: molecular weight marker 100 bp; C+: positive control; C-: negative control; 1-34: turtle samples).

That study analyzed free-living animals from a stream located in an urban environment, which may have increased the probability of encountering the pathogen, as such an environment comprises rodents and other animal species that provide favorable conditions for *Leptospira* spp. to remain alive for long periods (Ebani, 2017). The animals included in the present study lived in artificial lagoons, in which the water was not managed regularly; of note, all samples showed negative results for *Leptospira* spp. A possible explanation for this result may be because these species coexist in the aquatic environment for many years and, therefore, Testudines may have developed mechanisms to prevent infections by this bacterium, as suggested by Oliveira (2013).

Another possible explanation is that the animals included in this study might have come into contact with the pathogen; however, by the start of the study, these animals might have eliminated

the pathogen from their bloodstream or the infection might have already progressed into the chronic phase, therefore rendering pathogen detection in the blood difficult. Further research is necessary to determine whether blood is an ideal biological material for the detection of *Leptospira* spp. infection in turtles. The results of this research contributed to new reports regarding the possible infection by *Leptospira* spp. in reptiles kept in captivity.

However, it is important to apply other methodologies, such as serological and physical examination, to determine the health of the animals, and implement control measures to prevent the possible transmission of these zoonotic agents to other animals and to professional caregivers/caretakers, thereby avoiding possible damage to public health.

Keywords: testudines, *Leptospira* spp., reptiles, Amazon, public health

RESUMO

Existem poucos estudos sobre doenças infecciosas em animais silvestres. O objetivo deste estudo foi pesquisar DNA de *Leptospira* spp. em sangue de tartarugas mantidas em cativeiro, pertencentes ao Bosque Rodrigues Alves (Jardim Zoobotânico da Amazônia). O DNA foi isolado das amostras de sangue coletadas de 148 tartarugas pertencentes a seis espécies diferentes. A reação em cadeia da polimerase (PCR) foi realizada utilizando-se iniciadores específicos para DNA de *Leptospira* spp. Nenhuma das amostras apresentou resultado positivo para *Leptospira* spp.

Palavras-chave: testudines, *Leptospira* spp., répteis, Amazônia, saúde pública

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