



Incidence of leptospirosis in non-human primates at the santarém zoo, Pará, Brazil

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ABSTRACT. Leptospirosis is an infectious disease that affects practically all domestic and wild animals, which can become carriers and, as a consequence, will contribute to the spread of the microorganism in nature. The present study aims to detect the occurrence of leptospirosis against by the microscopic serum agglutination test in animals at the Zoo of the Centro Universitário da Amazônia (ZOOUNAMA). 26 adult animals of both sexes and of the following species: 9 *Sapajus apella*, 2 *Cebus kaapori*, 3 *Ateles marginatus*, 10 *Ateles paniscus*, 1 *Chiropetes satanas*, 1 *Allouata belzebul*. Of the animals studied, 3 (11.5%) were reagents to be taken against *Leptospira* spp. The serovars found in the different species were: *Icterohaemorrhagiae*, *Balum*, *Hardjo* and *Grippothyphosa*. SAM proved to be efficient to detect against leptospira in non-human primates. *Icterohaemorrhagiae* type serum occurred with research results showing that it is also important in leptospirosis in non-human primates.

Keywords: antigen; epidemiology; *Leptospira*.

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Introduction

Leptospirosis is an infectious disease that affects practically all domestic and wild animals, the most affected being carnivores, rodents, primates and marsupials that can become carriers and, as a consequence, will contribute to the spread of the microorganism in nature, but rodents make up for one of the main reservoirs of spirochetes, transmitting the disease to humans and different species of animals (Oliveira, Arsky, & Caldas, 2013; Freitas, Seleski, Menezes Chalkidis, Silva, & Junior, 2021; Souza Júnior et al., 2006; Teles et al., 2023). Despite the efforts of professionals to keep up with strict sanitary management, the zoo environment continues to be suitable to the spread of a range of diseases, many of them zoonotic (Basso et al., 2022; Galarce et al., 2021; Santos et al., 2021).

In the primate order, the distribution and occurrence of anti-*Leptospira* antibodies have already been identified in some studies and these findings showed that primates are exposed to several *Leptospira* serovars, but research on the epidemiology of leptospirosis in free-living primates is still scarce, considering the diversity of primates existing in Brazil (dos Santos et al., 2023; Esteves et al., 2005; Gonçalves et al., 2021). From research related to the most relevant studies in the literature on the topic, it can be inferred that the low incidence of leptospirosis in nature is associated with the behavior and arboreal habits of neotropical primates (Oliveira et al., 2013; Reid, Herron, Hines, Orchard, & Altman, 1993; Wilson et al., 2021).

Some studies carried out in the North of Brazil have shown positive reactions for the species of neotropical primates *Cebus apella*, *Alouatta caraya*, *Nasua nasua* and *Cerdocyon thous* (Grumann et al., 2017; Lima et al., 2014). However, studies on *Leptospira* in wild animals in the region are still very limited. The objective of this work was to investigate the prevalence of leptospirosis in non-human primates kept in captivity at the Santarém Zoo, as well as to identify possible serotypes present by microscopic agglutination method with live antigens.

Material and methods

This work was developed in the Zoo of the University Center of the Amazon (ZOOUNAMA), installed in an area of 147 hectares, given by a lending contract by the Ministry of Defense / Army / 8th BEC, located in the municipality of Santarém, state of Pará, and its squad it consists of 7 species of primates.

Twenty six adult animals of both sexes and of the following species were used: nine *Sapajus apella*, two *Cebus kaapori*, three *Ateles marginatus*, ten *Ateles paniscus*, one *Chiropetes satanas*, one *Allouata belzebul* (Table 1).

Table 1. Distribution of species according to scientific name, popular name, males and females.

Scientific name	Popular name	Males	Females	Total
<i>Sapajus apellas</i>	Macaco prego	5	4	9
<i>Cebus kaapori</i>	Caiarara	0	2	2
<i>Ateles marginatus</i>	Macaco aranha da testa branca	0	3	3
<i>Ateles paniscus</i>	Macaco aranha da testa vermelha	5	5	10
<i>Chiropetes satanas</i>	Macaco judeu	0	1	1
<i>Allouata belzebul</i>	Guariba	0	1	1
Total	-	10	16	26

Between June and July 2014, following the handling and routine measures of ZOOUNAMA, whole blood samples were collected from 26 adult individuals (males and females), distributed in 7 species of monkeys. The animals were physically restrained with the aid of handcuffs for later intramuscular administration of 10 mg kg⁻¹ of ketamine and 0.5 mg kg⁻¹ of xylazine (Viana, 2007) (Alfonso et al., 2020; Ferreira et al., 2020; Koo et al., 2019; Viana, 2007).

Blood was collected through venipuncture (saphenous vein), using disposable 5 mL syringes and a 25x7 mm hypodermic needle. The samples were placed in tubes without anticoagulant, and kept at a temperature of +2 to +8°C. After collection, the whole blood was separated from the serum by centrifugation for five minutes the 3.000 rpm at the Laboratory of the Veterinary Planeta Animal Laboratory, the serum obtained was placed in properly identified 1 mL tubes and kept refrigerated at -20°C until the exam was performed.

The microscopic agglutination test (SAM) is performed with the use of live antigens which, in addition to detecting specific antibodies, is also used in the identification and classification of isolated serovars. Antibodies can be revealed by agglutination with serum collected between the 8th and 10th day after the onset of leptospiremia and reach a maximum titer around one month (Danielle et al., 2021; Jayasundara et al., 2021; Verma et al., 2020).

In SAM, the antibodies present in the serum react with the epitopes of lipopolysaccharides (LPS), present on the surface of the leptospires, and the antigens used are cultures of standard strains of leptospires, maintained by weekly peaks in liquid: Stuart, Ellinghausen or similar. Only cultures from 4 to 14 days of cultivation that do not contain contaminants or self-agglutination should be used as antigens. The collection of antigens used in the microscopic agglutination test must include representatives of the serogroups of all serovars existing in the country or region for the species in question (Karpagam & Ganesh, 2020; Krecic, 2018; Samrot et al., 2021).

The serum was sent to the Hermes Pardini Institute Laboratory where serology was performed using the microscopic agglutination serum method with a collection of live antigens that included 24 serological variants of leptospires: Andamana, Australis, Autumnalis, Bataviae, Balun, Canicola, Castellonis, Celledoni, Cynopteri, Copenhageni, Djasiman, Grippothyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Panama, Patoc, Pomona, Pyrogenes, Sejroe, Shermani, Tarassovi Wolfii.

A cross-sectional epidemiological study was carried out, since exposed and sick animals were studied at the same time.

The animals were considered reactive when they had titrations equal to or greater than 1:100. The data were analyzed using the Microsoft Excel® 2014 program, considering the sex and species of primates that showed positivity.

Results and discussion

This work represented the first serological research on the prevalence of anti-leptospira antibodies in non-human primates at the zoo ZOOUNAMA in the town of Santarém.

Of the 26 animals analyzed, 3 (11.5%) were positive for leptospirosis and the serovars found were Icterohaemorrhagiae, Balun, Hardjo and Grippothyphosa (Figure 1).

A similar study was carried out in Aracaju-Sergipe where it found positive primates for *Leptospira*, of the 18 primates analyzed 2 (11.1%) were positive for leptospirosis, and reports that shows the serovar Icterohaemorrhagiae is found in synantropic rodents as maintenance hosts, as well demonstrating that other

species may have participated in the epidemiological chain of this sip (Pimentel et al., 2009). The rodents end up spreading the serovar through the urine and thus affecting other species of animals (Esteves, Neto, Girio, Vergara, & Carvalho, 2005; Murillo et al., 2020; Piredda et al., 2023), but another study shows that these rodents, as they are not a common part of this environment, can circulate at night and thus contaminate animals, which may explain its occurrence in the studied primates (Chuma et al., 2020; Ferreira et al., 2011; Rajeev et al., 2020).

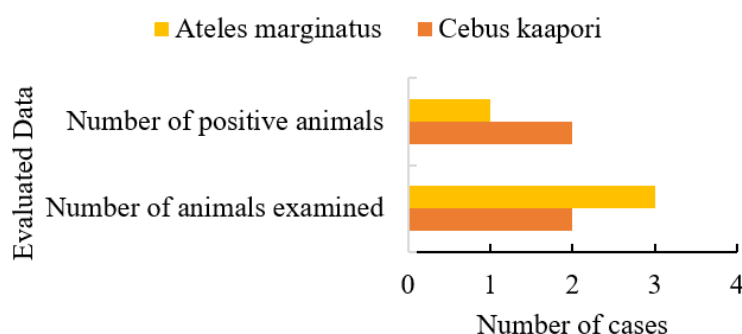


Figure 1. Results of exams by SAM test according to species, number of animals examined, numbers of positive animals and serovars found.

Among the studies carried out with primates using SAM as a diagnostic method, there is a study in the state of Tocantins in 2002 where a search for antibodies against *Leptospira* was carried out with 286 blood samples *Cebus apella* (capuchin monkey). Of these animals 46 were positive for the serovar Pomona, Brasiliensis, Mini, Swajizak, Grippothyphosa, Sarmin, Fluminense, Autumnalis, Hebdomadis, Guaratuba, Javanica and Icterohaemorrhagiae, of the 82 samples of *Alouatta belzebul* (guariba monkey), 2 were positive for the mangus serovars (Junior et al., 2006) (Souza Júnior et al., 2006).

From 1996 to 1999, a serological survey for leptospirosis was carried out in wild animals kept in captivity at the Fundação Parque Zoológico de São Paulo, in which of the 302 serological samples analyzed, 19.5% (59/302) were positive for the SAM test. Regarding primates, the analysis of the results showed 25 positive animals (22.5%), out of a total of 111 animals examined (Corrêa et al., 2004).

At the National Primate Center (CENP), Ananideua - Pará, a study was carried out in which 84 sera from 11 species of neotropical primates were used for the SAM test for antibodies against *Leptospira* spp, of which 35 had anti-leptospira antibodies and 49 were seronegative (de Lima Brasil et al., 2013; Molina et al., 2019).

Another study of great importance was carried out in Mata de Santa Teresa, Ribeirão Preto, São Paulo, with free-living *Cebus apella nigrinus*. With the use of SAM as a diagnostic method with 55 animals and positive serum reaction was observed in 33 animals (60%). The serovars found were Shermani, Andamana, Pyrogenes, Grippytyphosa, Australis, Canicola, Castelonis, Copenhageni, Hardjo, Hebdomadis, Pomona and Wolffi (Girio et al., 2021; Silva et al., 2013).

In the Northeast region, the occurrence of antibodies against *Leptospira* in primates of the genus *Cebus* kept in captivity was studied, where 139 blood serum samples were analyzed using the SAM technique, with anti-*Leptospira* antibodies, serovar Patoc in 6.5% of primates (Ferreira et al., 2011; Oliveira & Santos, 2023).

Conclusion

The microscopic agglutination test was effective to determine *Leptospira* infection in non-human primates, with the serovars Icterohaemorrhagiae, Ballun, Grippothyphosa and Hardjo being identified in this study, proving, for the first time, the occurrence of such infection in non-human primates belonging to the zoo in the municipality of Santarém, Pará.

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