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Occurrence of antibodies to *Leptospira* spp and *Toxoplasma gondii* in captive wild animals in the Zoobotanical Park of Petrolina, PE, Brazil

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ABSTRACT

OBJECTIVE

Leptospirosis and toxoplasmosis are cosmopolitan zoonoses affecting domestic and wild animals that can inhabit urban environments acting as reservoirs of infectious agents, increasing the risk of zoonotic diseases transmission to humans. The present study aimed to detect serum antibodies to *Leptospira* spp. and *Toxoplasma gondii* in captive wild animals from the Zoobotanical Park, located in the city of Petrolina, State of Pernambuco, Brazil.

METHODS

Samples were collected from 72 wild animals, including 12 mammals, 26 birds, and 33 reptiles. Serological diagnosis for anti-*T. gondii* antibody detection was performed on birds and mammals using the modified agglutination test (MAT Toxo). Anti-*Leptospira* spp. antibodies were detected in reptiles and mammals by microscopic agglutination test (MAT Lepto).

RESULTS

The occurrence of *T. gondii* antibodies was 56.4% (22/39), with positive animals from the genera: *Procyon cancryvorus* (1/2), *Ara ararauna* (4/4), *Patagioenas picazuro* (5/5), *Amazona aestiva* (5/5), *Aratinga acuticaudata* (3/3), *Tayassu tajacu* (2/2), N*asua nasua* (1/1) and *Cerdocyon thous* (1/1). Anti-*Leptospira* spp. antibodies were found in 4.4% (2/45) of the animals, with one out of the two *Tamandua tetradactyla* positive for the subgroup *Australis* and one out of the 23 *Geochelone carbonaria* positive for the subgroup *Hebdomadis*.

CONCLUSIONS

Captive wild animals from zoos can take part in the life cycle of these agents, acting as reservoirs, thus assuming an important role in the epidemiological chain of these important zoonotic agents.

DESCRIPTORS

Leptospirosis, Toxoplasmosis, Zoo, Zoonoses, Conservation veterinary medicine.

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INTRODUCTION

The current global situation and environmental problems caused by anthropogenic actions, have negative effects in the biodiversity[1]. Zoos act as a solution by attracting the public, and the modern Zoos are used as educational tools, sensitizing people to seek a change in behavior, aiming to promote the conservation of biological diversity[1,2]. Wild animals kept in zoos are susceptible to various environmental imbalances, and they are in contact with other wild, domestic, and synanthropic animal species[3]. Wild animals that live in the urban environment can act as reservoirs of infectious agents. As zoological parks are usually located in urban areas, the spread of infectious agents from free-living wild animals to other animals in captivity is possible[4].

Leptospirosis, caused by bacteria of the genus *Leptospira*, is considered a zoonosis of worldwide distribution with several wild animal species considered reservoirs for its spread[5]. *Leptospira* spp. penetrates through intact or damaged skin in the mucous membranes and multiplies in the blood, practically in all organs and tissues[6]. Rodents are main reservoirs, and the bacteria usually multiply in the kidneys, making urine the principal source of elimination, contaminating water, soil, and food[7].

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii*, a mandatory intracellular parasite, with a worldwide distribution[8]. The protozoan has a sexual cycle that occurs in felines (definitive hosts) and an asexual cycle in birds and mammals, including humans, as intermediate hosts[9]. *T. gondii* has tachyzoites, bradyzoites, and oocysts as infective forms[10]. Carnivorous or omnivorous hosts, including humans, are potentially infected by bradyzoites in meat, sporulated oocysts in contaminated water or food, or intrauterine by tachyzoites[11,12]. Herbivores can be infected via oocysts in soil, water, or uncooked vegetables[13].Currently, studies on the transmission chain of zoonosis include not only domestic animals but also wild animals[14]. Wild animals play an important role as reservoirs or carriers for these diseases in nature and captivity[15], however, few serological investigations have been carried out in animals that are in captivity, including those in zoos, breeding, and research centers[16,17,18,19]. Thus, the present study aimed to perform a serological survey of *Leptospira* spp. and *T. gondii* in wild animals from the Zoobotanic Park, Petrolina, Pernambuco (PE).

METHODS

Study Location

The study was performed at the Zoobotanical Park of the 72nd Motorized Infantry Battalion, located in the municipality of Petrolina (S 09º 23' 79", W 40º 28' 86"), PE, Northeastern Brazil. Created in 2007, this is the only Zoobotanical Park in the Middle São Francisco region, occupying an area of 10,000 m2. The squad consists of 135 animals from apprehensions, carried out by the Brazilian Institute of the Environment of Natural Resources (IBAMA), military police, and captured by the fire Department of the cities of Petrolina (State of Pernambuco) and Juazeiro (State of Bahia). Approximately 90% of species are from the Caatinga Biome, and 10% are species from other Brazilian biomes. The enclosures are built of masonry and steel mesh complying with the conformity of the Normative Instruction of IBAMA, using tall trees to reduce the temperature in the environment. The Zoo also serves as a source of leisure for communities in the surrounding cities and contributes decisively to the environmental education of people from all ages, and assisting in the training of Caatinga army fighters.

Blood Collection

Blood samples were obtained from 70 animal specimens from different classes: birds, reptiles, and mammals, divided into 24 species. Two synanthropic animals found in the location during the collection of the samples were included in this study, being an opossum (*Didelphis albiventris*) and a black rat (*Rattus rattus*), totaling 72 sampled animals.

The sample collection was conducted in three visits, attending the classes of birds, reptiles, and mammals. For the procedure, physical and/or chemical restraint was performed with the aid of nets, hand nets, leather scrape gloves, and pressing cage according to the species. The access of choice for blood collection, needle size, and syringes, as well as the amount of blood to be collected, was based on the species and body mass of each animal.

The blood samples were stored in tubes with anticoagulants, homogenized, identified, refrigerated, packed in Styrofoam boxes, and sent to the laboratory where the samples were centrifuged at 5,000g for 15min to obtain plasma, which was subsequently transferred into 1.5mL microtubes and stored at -20ºC until serological tests were performed.

Detection of IgG anti-*T. gondii* antibodies

Serological examination by the Modified Agglutination Test (MAT Toxo) was performed on samples from birds and mammals following the protocol of Dubey and Desmonds[20].

Sera were diluted using a buffered solution (PBS, pH 7.2). Serial dilutions of 1:25, 1:50, and 1:500 were initially performed. The sera of the animals with titers greater than or equal to 50 were again two-fold serially diluted until reaching the maximum reaction titer. Then, 150µL of *T. gondii* tachyzoites fixed in formalin were mixed with 2.5mL of PBS (pH 8.5), 35 µL of 0.2 M mercaptoethanol, and 50µL of 0.2% Evans Blue; 25µL of this reaction solution was distributed in each well of a 96-well microplate with a U-shaped bottom. In sequence, the diluted sera were transferred to this microplate and homogenized with the reagents. The plate was sealed with adhesive plastic to prevent evaporation and incubated overnight at 37°C. The reading was performed according to Desmonts and Remington[21].

Detection of anti-*Leptospira* spp. agglutinins

Microscopic serum agglutination test (MAT Lepto) was performed, as recommended[22, 23]. To perform MAT Lepto, live cultures of *Leptospira* spp. were used with each serogroup. Cultures were sown in modified EMJH liquid medium[24], supplemented with 15% sterile rabbit serum, then inactivated by a thermal process at 30°C for 30min, and enriched with a mix of 1% sodium pyruvate, 1% calcium chloride, 1% magnesium chloride, and 3% L- asparagine. The cultures were incubated at 28°C in a bacteriological incubator for a period of 7–10 days.

Each serum sample was diluted 1:50 in Sorensen's buffered saline. From this dilution, 50µL was distributed in a 96-well U-shaped bottom microplate, with 50µL of each corresponding antigen added to achieve a new 1:100 dilution. All samples were tested with an antigenic battery of 24 serological variants (serovars). The microplates were shaken and incubated at 28°C for 3h in a bacteriological incubator. The reaction was read in an optical microscope (Jena Zeiss) with a dark field condenser (MCE), operated with an objective (Epiplan) 20x /0.2 and ocular 10 at 200×magnification to evaluate the degree of agglutination.

Ethical aspects

This project followed the Ethical Principles of the Brazilian

Institute of the Environment and Renewable Natural Resources - IBAMA (protocol No. 2611.2394/2009-PE) and the Ethics Committee in the Use of Animals at the Federal University of Vale do São Francisco (protocol No. 0021/220515).

RESULTS AND DISCUSSION

Through questionnaires applied by the administration of the Zoobotanical Park, it was verified that of the 72 animals used for sample collection, 64 were captured by IBAMA, six by the Fire Department, and two were apprehended by the local military police. These animals received a diet based on fruits and vegetables obtained at the Supply Center (CEASA) in the city of Juazeiro, Bahia. The meat was donated by the Health Surveillance and viscera from the Municipal Slaughterhouse. Grains were obtained from local stores. The processing of these foods was carried out by trained professionals meeting the sanitary requirements, and the water offered to these animals was potable. It was observed the presence of domestic animals, such dogs and cats, as well as synanthropic animals, in the facilities of the Park. Two synanthropic animals, a *Didelphis albiventris* and a *Rattus rattus* were captured and included in this study.

Infection and spread of pathogens in zoos can involve captive animals, synanthropic animals, employees, and the visiting public[25]. Some serological surveys have demonstrated the involvement of wild species in the epidemiology of leptospirosis and toxoplasmosis, but captive populations of wild animals are poorly studied, particularly in Brazil.

The Caatinga Biome has a fauna that is adapted to challenging climatic conditions, resulting in an environment with a high rate of endemism[26]. Wild animals are widely used in this region for subsistence (food), breeding (pets), and even for medicinal and magical religious uses[27,28]. Occurring exclusively in Brazil, this biome has its main area located in the Northeast region, presenting a small stretch in the northern portion of the state of Minas Gerais, with a semi-arid climate, presenting less than 800 mm of annual precipitation, totaling 734,000 km[29]. In the Caatinga, environmental degradation is intense[30], particularly in riparian forest environments where vegetation is frequently removed to establish agricultural areas, mainly irrigated.

The municipality of Petrolina, PE, in the Brazilian semi-arid region, São Francisco Valley, represents a medium-sized municipality in a degraded environment, favoring the circulation of *T. gondii*[31].The circulation of *Leptospira* spp. in wild, free-living animals has already been reported in unserved rural areas in this municipality[32].

In the present study the occurrence of anti-*Leptospira* spp. antibodies in the animals were 4.4% (2/45). One out of the 12 mammals (8.3%) and one from the 33 reptiles (3.0%) examined were reagent (Table 1). The mammal *Tamandua tetradactyla* (Southern tamandua) was positive for the subgroup Australis and the reptile *Geochelone carbonaria* (Red-footed tortoise) for the subgroup Hebdomadis, representing the most likely serovars Australis and Hebdomadis, respectively.

A serological study of leptospirosis in reptiles, birds, and mammals is of fundamental importance because scarce information on the disease behavior is available in these species. Using serological surveys, it is possible to verify whether these animals had contact with leptospirosis and whether there was a multiplication of the agent in the organism. However, the type of immune response they develop is not known, nor whether MAT leptin standardization is validated for them, so the results found, even with low titers, are essential[3].

The few studies carried out with the group of reptiles pointed to a possible role of leptospirosis reservoirs and showed that even healthy animals had anti-*Leptospira* antibodies[33]. The predominance of low anti-*Leptospira* spp. antibodies has been associated with infection in reptiles[34,35,36]. Nevertheless, Fornazari[37] highlights the scarcity of studies on the presence of antibodies and the lack of information about the role of reptiles in the transmission of the agent. In Brazil, DNA detection in turtles[36] and snakes[35] has been reported.

In the present study, a specimen of red-footed tortoise (*Geochelone carbonaria*) was reactive to the serovar Hebdomadis; this is the third description on this species. Silva et al[3] conducted a serological survey for leptospirosis in the city of Ribeirão Preto, State of Sao Paulo, Brazil, in specimens of the Family Testudinidae, and found eight *G. carbonaria* (08/29) reagents for serovars Patoc (05/08), Hebdomadis (02/08), and Canicola (01/08). Another report also was made by Esteves et al[38] in *Geochelone* spp., who analyzed 16 samples and found only one positive for the Andamana serovar.

Another species that was reactive for anti-*Leptospira* antibodies was *Tamandua tetradactyla* (collared anteater) for serovar Australis. Previously, Souza Júnior et al[39] and Lilenbaum et al[40], respectively in the States of Tocantins and Rio de Janeiro, Brazil, have reported the serovar Icterohaemorrhagiae for this animal species.

The results from the evaluation by MAT Lepto corroborate with a study conducted at the Chapultepec Zoo, in Mexico City, where the serovars Icterohaemorrhagiae, Canicola, Pyrogenes, and Hebdomadis, representing the serogroups Icterohaemorrhagiae, Canicola, Pyrogenes, and Hebdomadis were described[41] and differed from the findings of Brazil et al[14], with the captive fauna of the Zoobotanic Park Arruda Câmara, in the city of João Pessoa, Paraíba, Brazil, in which only one ocelot (*Leopardus pardalis*) from the 49 animals examined, was seropositive for serovar Icterohaemorrhagiae.

Corrêa et al[42] performed a serological survey for leptospirosis in 302 captive animals at the Zoo from the Municipality of São Paulo, Brazil, and 59 (19.5%) were reagents for MAT Lepto. Also, in the state of São Paulo, in wild animals at the Municipal Zoo of Ribeirão Preto, 388 samples were analyzed, and the most frequent serovars found were Patoc, Andamana, Canicola, Icterohaemorrhagiae and Panama[3]. From the 388 samples, 339 sera were collected from captive animals and 92 (27.1%) were reagents, being found serovars Patoc, Andamana, Canicola, Icterohaemorrhagiae and Panama. The other 49 sera were collected from free-living animals and 11 (22.4%) were reagents, presenting the serovars Patoc, Autumnalis, Copenhageni, Pyrogenes and Australis.

Table 1 shows the species of birds and mammals positive for anti-*T. gondii* antibodies. From the 39 animals analyzed, 21 (53.8%) were seroreagents, corresponding to 17 of the 26 birds (65.3%) and five out of the 12 mammals (42%). The bird's species found to be seroreagent for *T. gondii* were: *Patagioenas picazuro* (5/5), *Ara ararauna* (4/4), *Aratinga acuticaudata* (3/3) and *Amazona aestiva* (5/5), and the positive mammal's species were: *Tayassu tajacu* (2/2), *Cerdocyon thous* (1/1), *Nasua nasua* (1/1) and *Procyon cancryvorus* (1/2).

Table 1. Serodiagnosis of *Leptospira* spp. by Microscopic Agglutination Test (MAT 570 *Leptospira*) in mammals and reptiles and for *Toxoplasma gondii* by Modified Agglutination Test (MAT *Toxoplasma*) in birds and mammals from Zoobotanical Park, Petrolina, State of Pernambuco, Brazil.

Class	Order	Species	Popular name	MAT *Toxoplasma* N. positive/N. tested (%) titer	MAT *Leptospira* N. positive/N. tested (%) titer	*Leptospira* Serogroup
Birds (N=26)	Cariamiformes	*Cariama cristat*	Red-legged seriema	0/2 (0)	*	-
	Columbiformes	*Patagioenas picazuro*	Picazuro pigeon	5/5 (100) 25	*	-
	Falconiformes	*Caracara plancus*	Southern crested caracara	0/5 (0)	*	-
	Psittaciformes	*Ara ararauna*	Blue-and-yellow macaw	4/4 (100) 100	*	-
	Psittaciformes	*Ara chloropterus*	Red-and-green macaw	0/1 (0)	*	-
	Psittaciformes	*Aratinga acuticaudata*	Blue-crowned parakeet	3/3 (100) 25	*	-
	Psittaciformes	*Amazona aestiva*	Turquoise-fronted amazon	5/5 (100) 25	*	-
	Psittaciformes	*Aratinga jandaya*	Jandaya parakeet	0/2 (0)	*	-
Mammals (N=12)	Artiodactyla	*Tayassu tajacu*	Collared peccary	2/2 (100) 50,100	0/2 (0)	-
	Carnivora	*Cerdocyon thous*	Crab-eating fox	1/1 (100) 25	0/1 (0)	-
	Carnivora	*Nasua nasua*	South American coati	1/1 (100) 25	0/1 (0)	-
	Carnivora	*Procyon cancryvorus*	Raccoon	1/2 (50) 100	0/2 (0)	-
	Cingulata	*Euphractus sexcintus*	Six-banded armadillo	0/1 (0)	0/1 (0)	-
	Didelphimorphia	*Didelphis albiventris*	White-eared opossum	0/1 (0)	0/1 (0)	-
	Pilosa	*Tamandua tetradactyla*	Collared anteater	0/2 (0)	1/2 (50) 100	Australis
	Primates	*Alouatta caraya*	Black howler monkey	0/1 (0)	0/1 (0)	-
	Rodentia	*Rattus rattus*	Black rat	0/1 (0)	0/1 (0)	-
Reptiles (N=33)	Crocodilia	*Caimam latirostris*	Broad-snouted caiman	*	0/2 (0)	-
	Squamata	*Bothrops erythromelas*	Bothrops erythromelas	*	0/1 (0)	-
	Squamata	*Crotalus durissus*	Rattlesnake	*	0/1 (0)	-
	Squamata	*Epicrates cenchria*	Rainbow boa	*	0/1 (0)	-
	Squamata	*Python reticulata*	Python	*	0/1 (0)	-
	Testunidae	*Geochelone carbonaria*	Red-footed tortoise	*	0/23 (0)	-
	Testunidae	*Geochelone denticulata*	Yellow-footed tortoise	*	1/4 (25) 100	Hebdomadis

*not tested; N = number of animals tested

The total occurrence in mammals and birds observed in the present study (53.8%) was lower than that reported by Feitosa et al[43], that was 40.5% (62/153) in captive animals from Joao Pessoa, Paraíba, with all South American coatis (Nasua nasua) examined (n= 5) being positive for *T. gondii* antibodies. Pimentel[15], also in a Zoo in the Northeast region of Brazil, State of Sergipe, examined samples of coatis (*N. nasua*), raccoons (*P. cancrivorus*) and crab-eating fox (*Cerdocyon thous*), obtaining 100% (2/2), 66.6% (4/6), and 0% (0/1) seropositivity, respectively. Carneiro et al[44] analyzed samples of captive coatis from Goias and found an occurrence of 43% (7/16). Maia et al[45] examined a larger sample (n=99) of coatis from Parque Ecológico do Tietê (occurrence of 43% positive animals with titers ranging from 50 to 3,200). All these studies indicate a relatively high occurrence of *T. gondii* antibodies in this species in Brazilian Zoos.

A study performed at the Zoological-Botanical Park, João Pessoa, in Paraiba, found *T. gondii* antibodies in Collared peccary (*Tayassu tajacu*) (1/1), presenting a titer of 400, and in a Black rat (*Rattus Rattus*) (1/1) with a titers of 400[43]. In a study conducted in Fernando de Noronha Archipelago, Pernambuco, it was found a seroprevalence of 38.2% (13/34) for *T. gondii* antibodies in wild black rats (*Rattus rattus*)[46], with titers ranging between 16-512. Carme et al[47], in French Guiana, also found antibodies in collared peccary (*T. tajacu*) (8/13), showing an occurrence of 62%.

Almeida et al[48] found a frequency of antibodies anti-*T. gondii* of 50% (4/8) and 29.41% (5/17), respectively for free-range and captive *Cerdocyon thous* in Northeast Brazil.

The infection by *T. gondii* in captivity animals can occur as a result of factors such as stress associated with captivity, the presence of synanthropic animals, and possible mistakes in health management, such as feeding carnivores with meat that has not been properly frozen[49].

In the examined birds the occurrence of antibodies anti-*T gondii* was 65.3% (17/26) with four *Ara ararauna*, five *Patagioenas picazuro*, five *Amazona aestiva*, and three A*ratinga acuticaudata* reactives. In Northeast Brazil, the species *P. picazuro* is of great importance, as it serves as a source of food for humans and could participate in the transmission of toxoplasmosis in this region.

T. gondii has been described in pigeons and other birds since the beginning of the last century[50], when Carini[51], in Brazil, observed the parasite in smears of the liver and spleen from a dove of rock (*Columba livia*). Birds are important in the life cycle and epidemiology of this coccidia, mainly because their tissues represent important sources of protein in the feeding of felids and humans[52].

The occurrence of toxoplasmosis in Psittaciformes is uncommon, being reported in postmortem diagnosis of Australo-Asian species[53,54], with no record of serological diagnose in the literature. In this study 57.69% of the birds studied were from the Order Psittaciformes (15/26), which 80% (12/15) were positive for *T. gondii*. Inside this order, a study was performed in Pernambuco state[55] for detection of *T. gondii* antibodies in the same species searched in the present study, except for *Aratinga acuticaudata*. As far as the authors knowledge goes, this is the first study that found antibodies for *T. gondii* in *A. acuticaudata*. Raptors are considered refractory to *T. gondii*[45], but the occurrence of toxoplasmosis in Strix varies[56] is described in *Haliaeetus leucocephalus*[57]. Gonçalves et al[58] found 12 (15.2%) of the 79 wild birds, including the species parrot (*A. aestiva*) seropositive for *T. gondii*. Gennari et al[59] found antibodies in 73 (36.1%) of 202 wild birds from the Atlantic Forest, São Paulo, Brazil.

Few are the seroepidemiological studies of *T. gondii* infection in wild birds in Brazil. In the same region of the present study, three serological surveys were carried out. Gondim et al[60] found only one of the 167 (0.59%) examined free-living sparrows (*Passer domesticus*), caught near squares, positive to *T. gondii* antibodies. In the Agreste region, 60.3% (91/151) of sparrows from poultry farms were seropositive[57], and in the Fernando de Noronha Archipelago, 79.7% (157/197) of the free-living cattle egrets (*Bubulcus ibis*) were reagent to *T. gondii* antibodies[61].

Wild birds can be bioindicators for the presence of *T. gondii* in a given region, as the identification of seropositive birds suggests that they have ingested sporulated oocysts in the soil, water, or contaminated food[59], or in the case of birds of prey, ingestion of *T. gondii* tachyzoites or bradyzoites in cysts from prey tissues[25]. Thus, the occurrence of anti-*T. gondii* antibodies in seropositive birds can indicate the presence of definitive hosts, cats and other wild felids in the environment[25, 62].

CONCLUSION

T. gondii and *Leptospira* spp. are circulating at the Zoobotanic Park of Petrolina. The wild animals in the Park have been ap-

prehended from the same region and it is impossible to affirm whether they arrived infected or got the infection in the Zoo, however the presence of antibodies in this population of wild animals in an ex situ environment can suggest the circulation of both agents in the Caatinga. The results serve as a warning for the risk of a possible transmission of these pathogens between captive and stray animals, as well as for humans, and reinforce the importance of the one health' approach.

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