

SEROLOGICAL AND MOLECULAR DIAGNOSIS OF *Toxoplasma gondii* IN NON-HUMAN PRIMATES IN A ZOO

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ABSTRACT

The participation of wild animals as reservoirs or carriers of zoonoses in the wild and captivity is an emerging concern due to the potential for transmission of zoonoses such as toxoplasmosis. This study examined risk factors associated with the prevalence of *Toxoplasma gondii* in the infection of zoo primates. Serum samples from 43 primates were tested for *T. gondii* antibodies using two serological techniques: the Modified Agglutination Test (MAT), Immunofluorescence Antibody Test (IFAT; and one molecular technique: Polymerase Chain Reaction (PCR). Antibodies were found in 37.2% (16/43) of the animals. Among the species, 8/43 were seroreagents according to all three techniques and 8/43 only by the) IFAT. No sample was positive according to PCR. The risk factors studied, namely sex, age and category (Old World or Neotropical), were not significant in the statistical analysis ($p < 0.01$). However, other risk factors, such as the food and water supplied and the sand present in the animals' enclosures, were not analyzed, but have been shown in other studies to be potential causes of high prevalence of antibodies of animals surveyed in zoos. Hence, there is a need for regular monitoring of infection through periodic serological tests, to prevent infection of zoo workers and the visiting public.

Keywords: toxoplasmosis, captive primates, serology, risk factors

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DIAGNÓSTICO SOROLÓGICO E MOLECULAR DE *Toxoplasma gondii* EM PRIMATAS NÃO HUMANOS EM PARQUE ZOOLOGICO

RESUMO

A participação de animais selvagens como reservatórios ou portadores de zoonoses na natureza e em cativeiro é uma preocupação emergente devido ao potencial de transmissão de agentes zoonóticos, tais como a toxoplasmose. O estudo verifica fatores de risco associados com a prevalência de *Toxoplasma gondii* na infecção de primatas procedentes de zoológico. Amostras de soro de 43 primatas foram testadas para anticorpos para *T. gondii* utilizando as técnicas sorológicas teste de aglutinação modificada (TAM) e reação de imunofluorescência indireta (RIFI) ($cut-off \geq 25$) e a técnica molecular de reação em cadeia polimerase (PCR); anticorpos foram encontrados em 37,2% (16/43) dos animais, entre as espécies 8/43 foram sororreagentes para ambas as técnicas, 8/43 somente para TAM e 2/43 para RIFI. Nenhuma amostra se apresentou positiva na PCR. Os fatores de risco estudados como sexo, idade e categoria (velho mundo e neotropicais) não se apresentaram significativas à análise estatística ($p < 0,01$), entretanto, resultados indicam que fatores relacionados como alimentação e areia presente nos recintos dos animais não foram analisados, mas se mostraram em potencial, possível causa da alta prevalência de anticorpos dos animais pesquisados do Parque Zoológico, necessitando, portanto de um monitoramento constante para a infecção, pela realização periódica de testes sorológicos, como cuidados relacionados aos fatores de risco, como a procedência da areia utilizada nos recintos e a higienização adequada das frutas e verduras fornecidas aos animais como forma de prevenção da infecção para os funcionários bem como para o público visitante.

Palavras-chave: toxoplasmose, primatas de cativeiro, sorologia, fatores de risco

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RESUMEN

La participación de animales salvajes como reservorios o portadores de zoonosis en la naturaleza y en cautividad es una preocupación emergente debido al potencial de transmisión de agentes zoonóticos, como la toxoplasmosis. Este estudio verifica los factores de riesgo asociados a la prevalencia de la infección por *Toxoplasma gondii* en primates de zoológico. Se analizaron muestras de suero de 43 primates para detectar anticuerpos contra *T. gondii* mediante las técnicas serológicas de prueba de aglutinación modificada (MAT) y reacción de inmunofluorescencia indirecta (IFI) (punto de corte ≥ 25) y la técnica molecular de reacción en cadena de la polimerasa (PCR); se encontraron anticuerpos en el 37,2% (16/43) de los animales, entre las especies 8/43 fueron serorreactivas para ambas técnicas, 8/43 sólo para MAT y 2/43 para IFI. Ninguna muestra fue positiva en PCR. Los factores de riesgo estudiados como sexo, edad y categoría (viejo mundo y neotropical) no fueron estadísticamente significativos ($< 0,01$), sin embargo, los resultados indican que los factores relacionados con la alimentación y la arena presente en los recintos de los animales no fueron analizados, pero mostraron ser una causa potencial de la alta prevalencia de anticuerpos en los animales encuestados del Parque

Zoológico, Por lo tanto, es necesario el monitoreo constante de la infección, a través de pruebas serológicas periódicas, así como cuidados relacionados con los factores de riesgo, como el origen de la arena utilizada en los recintos y la limpieza adecuada de las frutas y verduras suministradas a los animales como forma de prevenir la infección tanto para los empleados como para el público visitante.

Palabras-clave: toxoplasmosis, primates en cautiverio, serología, factores de riesgo

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a zoonotic protozoan with worldwide distribution. This pathogen disseminates in the environment through contact with feces of felines that have *T. gondii* oocysts. Transmission of toxoplasmosis, disease caused by *T. gondii*, occurs mainly by the ingestion of cysts or sporulated oocysts in water, contaminated food or raw or undercooked meat containing bradyzoites (1).

Environmental contamination by oocysts is relevant in the transmission of toxoplasmosis because a single feline can release more than 100 million non-sporulated oocysts (2). Elimination of oocysts in the feces of felines occurs from 3 to 10 days after infection, followed by sporulation in the environment to infect other warm-blooded animals (1).

Humans and several animal species are susceptible to toxoplasmosis. Non-human primates (NHP) stand out for their vulnerability to the disease. They typically develop acute and severe reactions, quickly leading to death (3).

It is important to carefully manage zoo animals to prevent their exposure to toxoplasmosis. Seroprevalence in NHP is lower (11.6%) in free-living animals compared to captive animals (59.6%) (4). In captive animals, the infection by *T. gondii* has particular relevance since several animal species die as a result of severe toxoplasmosis (5).

Therefore, the main objective of this study was to identify the frequency of toxoplasmosis in captive NHP and to compare the techniques Modified Agglutination Test (MAT), Immunofluorescence Antibody Test (IFAT) and Polymerase Chain Reaction (PCR) in the laboratory surveillance of toxoplasmosis.

MATERIAL AND METHODS

Animals and study area

Blood samples from 43 non-human primates belonging to a municipal zoo in Southeast Brazil were analyzed. The animals were kept in enclosures distributed in several areas of the zoo, with easy access of synanthropic animals and domestic cats.

The study was approved by the Biodiversity Authorization and Information System (SISBIO, protocol 38258896), the Chico Mendes Institute for Biodiversity Conservation (ICMBIO) and the Ethics Committee on the Use of Animals (CEUA) of the Faculty of Veterinary Medicine and Animal Science (FMVZ) of Sao Paulo State University (UNESP), located in Botucatu, São Paulo (protocol 139/2014).

Sample collection

Blood samples used evaluated were collected by venipuncture during preventive dental procedures. Blood samples were collected in two tubes, one with anticoagulant EDTA and one without anticoagulant, for PCR and serological testing, respectively.

Laboratory techniques

Modified Agglutination Test (MAT)

MAT was performed as proposed by Desmouts and Remington (6). Serum samples were diluted in flat-bottom microplates, by adding 120 μL of Phosphate-buffered saline (PBS) 0.01M pH 7.2 in the first well and 50 μL in the remaining wells. Then 5 μL of serum was added to the first well (25 serum dilution), and after homogenization with a micropipette, 50 μL was transferred to the next well, corresponding to a serum dilution of 50. The same procedure was repeated until the dilution of 400 (7, 8, 9, 10). Next, 25 μL of each serum dilution was transferred to a respective well in a V-bottom, microplate, followed by addition of 25 μL of 2-mercaptoethanol (0.2M), diluted in PBS 0.01M pH 7.2, to break the IgM immunoglobulin (Ig) bridges aiming at the subsequent detection of IgG only. Then, 50 μL of the antigen preparation, already adjusted to the ideal concentration for use, was diluted in borate buffer pH 8.7 and added to each of the wells used. After homogenization, the microplates were sealed with laminated paper and incubated in an oven at 37 °C for 12 hours (11). A sample was considered negative when a deposit of the suspension of parasites was observed at the bottom of the well in the form of a button or ring. On the contrary, positivity was coined if a film covering at least half of the well bottom was seen (12).

Immunofluorescence Antibody Test (IFAT)

IFAT was performed according to Camargo (13), using the commercial conjugate anti-monkey IgG (Sigma® Co. St. Louis, USA), diluted in specific proportions for detection of anti-*T. gondii* antibodies. Sera was diluted in PBS 0.01M pH 7.2, starting at dilution 25 and doubled until the final dilution was obtained. For screening of positive animals, a cutoff point from the 25 dilution in PBS pH 7.2 was established after incubation in a humid chamber at 37 °C for 30 minutes. After incubation, the slides were washed twice in the buffer solution (10 minutes each wash). After drying the slides, the anti-monkey IgG conjugate was added. This conjugate had been previously diluted in a buffer solution with pH 7.2 containing 0.02% Evans blue dye. The slides were incubated in a humid chamber at 37 °C for 30 minutes and washed twice for 10 minutes each. The slides were read with a fluorescence microscope with 40x objective. Positive dilution was considered when the *T. gondii* tachyzoites presented no clear green fluorescence in the cell membrane against the red background. Final dilution titer was considered the highest dilution of serum in which there was still complete fluorescence at the edges of at least 50% of the tachyzoites. Absence of fluorescence or only at the end of the parasites, known as polar fluorescence, was considered a negative reaction. Positive samples at this dilution were tested one more time at dilutions of 25, 50, 100, 200 and 400, thus obtaining the sample's final antibody titer.

Polymerase Chain Reaction (PCR)

PCR was performed according to Homan et al. (14). For extraction of *T. gondii* DNA, we used the commercial kit AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific®, USA), following manufacturer's recommendations. In the PCR amplification, each 0.2 ml tube received PCR buffer (50 mM MKCL, 20 mM Tris-HCL), 1.5 mM MgCl₂, 0.2 mM of dNTPs, 1 U of Taq1-polymerase (Platinum® Taq DNA Polymerase, Invitrogen®, USA), 10 μM of each primer, 1 μL of each sample tested and 8.3 μL of distilled water (PCR mix), finishing with 11 μL of PCR mix and 1 μL of the DNA extraction product. Primers used were TOX4 (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5

(CGCTGCACACAGTGCATCTGGATT), with 529 bp (GenBank no. AFI46527). After electrophoresis, the size of the amplified fragments was verified through visual comparison of molecular weight against the standard strains that served as positive controls.

STATISTICAL ANALYSIS

Demographics data were used to compute general descriptive statistics. Frequencies and statistical differences among demographics variables were calculated using GraphPad Prism 5.0 program (GraphPad Software, San Diego, California, USA, 2016). Agreement test of diagnostics test used was performed using the diagnostic and agreement statistics spreadsheet from dag_stat (15).

RESULTS

MAT results showed that 37.2% (n=16/43) of the animals had antibodies to *T. gondii*, with titer 200 observed in 31.2% of the animals, titers 100 and 400 in 18.7% of the animals, and 25 and 50 in 6.2% of the animals. In the IFAT, 23.3% (n=10/43) of the animals were reactive, presenting titer 25 in 40% of the animals, titer 200 in 30% of the animals, titer 100 in 20% of the animals, and titer 50 in 10% of the animals. Seropositive species were *A. caraya* (2 animals), *A. paniscus* (1 animal), *E. pata* (2 animals), *M. sphinx* (2 animals), *P. hamadryas* (1 animal) according to both serological techniques; *T. seniculus* (1 animal), *A. chamek* (2 animals), *A. marginatus* (2 animals), *L. Lagotricha* (1 animals), *M. sphinx* (1 animals) and *P. hamadryas* (1 animals) in the MAT; and *M. sphinx* (1 animals) and *P. hamadryas* (1 animals) in the IFAT.

Frequencies were different among sex, age, and class groups. Regarding sex, 50% of samples were positive for *T. gondii* in males and 28% in females (P-value=0.20) in the MAT, while in the IFAT, the positive rates were 27.8% for males and 20% for females (P-value=0.71). Frequency of seropositive animals by the MAT was 14.3% among animals under one year of age and 41.7% among animals over one year of age. In the IFAT, frequency was 28.6% among animals less than one year of age and 22.2% among animals over one year of age. Regarding the NHP classes, neotropical animals presented a frequency of 40.7% seropositive results while old world animals' frequency was 31.2% in the MAT. In the IFAT, seropositive results represented 18.5% of neotropical animals and 31.2% of old-world animals. (Table 1).

Table 1. Univariate analysis of risk factors for the frequencies of anti-*T. gondii* antibodies in NHP housed in a zoo.

| Factors | Frequency of IgG antibodies against <i>T. gondii</i> | | | | | | |
|----------------|--|------------------|----------------|----------|-------------------|----------------|----------|
| | n ^a | MAT ^b | f ^c | P-value* | IFAT ^b | f ^c | P-value* |
| Sex | | | | | | | |
| Male | 18 | 9 | 50.0 | 0.14 | 5 | 27.8 | 0.55 |
| Female | 25 | 7 | 28.0 | | 5 | 20.0 | |
| Age | | | | | | | |
| ≤1 year | 7 | 1 | 14.3 | 0.17 | 2 | 28.6 | 0.71 |
| >1 year | 36 | 15 | 41.7 | | 8 | 22.2 | |
| Classes | | | | | | | |
| Neotropical | 27 | 11 | 40.7 | 0.53 | 5 | 18.5 | 0.33 |
| Old World | 16 | 5 | 31.2 | | 5 | 31.2 | |

^aNumber of animals studied (n=43), ^bAmount of animals positive for MAT and IFAT antibodies (titer≥25), and ^cFrequency of positive animals in respect to their parent group. *Fisher's exact test, association with frequencies of *T. gondii* by MAT and IFAT. MAT= Modified Agglutination Test; IFAT: Immunofluorescence Antibody Test. Sao Paulo, Brasil. 2023.

There was no significant association between the risk factors (sex, age, and NHP class) and positive titers when adjusting frequencies for the total number of animals studied (n=43). The highest prevalence was found for gender, in which 50% of male animals were seropositive in the MAT, contrary to what was observed in the IFAT for the class variable, in which 31.2% of the old world NHP were seropositive. In conventional PCR, all blood samples were negative for detection of *T. gondii*.

Analysis of frequency of positive titers indicated that between the two serological tests used, MAT detected higher number of positive samples and also higher titers in comparison to the IFAT technique, which was better at detecting positive titers for samples that were reactive in the initial screening (Table 2).

Table 2. Frequency of anti-IgG *T. gondii* antibodies titers by MAT and IFAT techniques in NHP housed in a zoo.

| Technique | Titration of anti- <i>T. gondii</i> antibodies | | | | | |
|-----------|--|------|------|------|------|------|
| | 25 | 50 | 100 | 200 | 400 | NR |
| MAT (%) | 6.2 | 6.2 | 18.7 | 31.2 | 18.7 | 18.7 |
| IFAT (%) | 40.0 | 10.0 | 20.0 | 30.0 | - | - |

MAT= Modified Agglutination Test; IFAT: Immunofluorescence Antibody Test. NR = non-reagent. N=43 animals. Percentage of positive titers by titer dilution. Sao Paulo, Brasil. 2023.

Finally, results from diagnostic and agreement statistics indicated that the Kappa coefficient between both serological tests was 0.3534 (95% CI: 0.07-0.64), which indicated a fair agreement between such tests, considering IFAT as the criterion. The agreement between positive results was 0.54 (95% CI:0.31-0.77) and the agreement between negative results was 0.80 (95% ci:0.69-0.91).

DISCUSSION

Several studies have already reported the high susceptibility of Neotropical NHP to *T. gondii* infection and the death of these animals in captivity is frequent (16). The manifestation of the disease in Neotropical NHP occurs acutely and severely, progressing to death (3). Although mortality rates in new world NHP are undetermined, studies report that is similar to cases of the disease in immunocompromised humans (17).

In the present study, a frequency of 37.2% (n=16/43) was observed in NHP primates belonging to the Municipal Zoo of Bauru, São Paulo, using the MAT and 23.3% (n=10/43) using the IFAT. Most of the positive animals had positive titers of 200 in the MAT and 25 in the IFAT. The tests were based on the detection of antibodies of the IgG class anti-*Toxoplasma gondii*, and it was not possible to determine the timing of infection of the animals, which is why we only define the chronicity of the infection. Though blood samples evaluated by PCR showed negative results, which could suggest that the animals may not be suffering from acute infection at the time of collection, sampling of more time points would be needed to accurately suggest absence of acute toxoplasmosis infection.

Regarding gender and positivity, 50% (n=9/18) of males were seroreactive according to the MAT and 27.8% (5/18) in the IFAT. Higher rates of infection in males (60%) were also found in the Brazilian state of Sergipe (7), while lower rates (33.3%) were found in the state of São Paulo (18), and no association between sex was found in captive NHP in the Amazon region (5). Conversely, Cano-Terriza (19) found significantly higher seropositivity in females in Spain.

Regarding the age range of the animals, there was a higher frequency in animals older than 1 year in the MAT (41.7%) and in animals younger than or equal to 1 year of age in the

IFAT (28.6%), but this difference was not considered significant, similar to that obtained by da Silva et al. (18) in São Paulo and Minervino (5) in the Amazon region.

The primates in this study were categorized into two different classes, called Neotropical NHP and Old World primates, which are differentiated by size and habits.

T. gondii infection was found to be directly related with mortality of Neotropical primates, while Old World primates were found to be asymptomatic and resistance or susceptibility were not recognized (20). Seropositivity of Neotropical and Old World NHP species did not differ statistically. Of the 27 Neotropical primates, 11 (40.7%) were seroreagents in the MAT and 5 (18.5%) were seroreagents in the IFAT, while out of 16 Old World primates, 5 (31.2%) were seroreagents in the MAT and 5 (31.2%) were seroreagents in the IFAT. Ferreira et al. (10) also reported that Neotropical primates were highly susceptible to *T. gondii* infection.

Though there is no gold standard test for toxoplasmosis, specifically in NHP, the use of two serological techniques for surveillance have been reported in literature (21). IFAT has been identified as the most frequently used serological test. The agreement test performed on both tests indicated there was a fair agreement (Kappa coefficient= 0.3534) in the results from both tests and it was specifically powerful when comparing negative results, suggesting that MAT may have a similar specificity as IFAT.

Captive NHP can be important reservoirs of *T. gondii*, assuming the role of sentinels for identifying sources of contamination in zoo enclosures. Due to the results obtained, we suggest continuing the serological research for *T. gondii* in the zoo primates, if possible with the collection of paired samples, so that the newly infected animals can be identified and checked for an increase in titers with repeat testing. Serological analyses for toxoplasmosis could also be performed on other species found in the zoo, such as felines, which are definitive hosts.

The investigation of sporulated oocysts in water and food, as well as in the sand of the enclosures was not carried out in this study. However, it has a fundamental role in determining the epidemiological chain of transmission of this zoonosis to animals.

Information on the origin of the meat consumed by animals and the sand used in the environment of primates living in enclosures is indispensable, since these may have been the source of sporulated *T. gondii* oocysts infecting animals.

In the present study, some risk factors were observed that may have contributed to infection, such as the sand of the enclosures where the Old World primates are housed. These enclosures were built recently, and the sand used may have come from other enclosures, enclosures of felids, or even from places outside the zoo. Thus, the sand could have been contaminated with oocysts, allowing the infection of the animals. This information is of extreme importance in light of the occurrence of deaths in the enclosure of penguins, where infection was detected by the presence of *T. gondii* oocysts, in addition to cysts in brain tissue of a penguin specimen sent for pathological examination, carried out in the Pathology Laboratory of Sao Paulo State University (UNESP), Araçatuba Campus (personal communication from the zoo's veterinarian).

Another risk factor found for primates is the presence of synanthropic animals, such as small rodents and domestic cats, and even feral cats, which may have access to the enclosures, as well as birds. In all cases, these animals can have *T. gondii* cysts in their musculature, and primates can acquire infection when ingesting them (22, 23).

The diet of all animals consisted of fruits and vegetables and raw meat, as well a commercial feed product, which was stored in bulk. The water came from the municipal piped water system of Bauru, São Paulo. It was not possible to relate the variables studied statistically with the routes of transmission and sources of infection associated with the presence of seropositive animals at the zoo. However, primates with carnivorous habit may have become infected by eating raw meat containing cysts with bradyzoites, as well as by drinking water and eating vegetables and fruits contaminated with oocysts.

Accurate information about the origin of the food, as well as the sand that is placed in the enclosures, was not available from the zoo managers. However, the risk factors such as the origin of water and food, as well as the sand in enclosures, should be taken into account in the epidemiological analysis of the disease in non-human primates. Attention should be paid to assuring the good hygiene of food and water offered to animals and the possibility of implementing a form of heat or chemical treatment of the sand or other material used in enclosures, in order to destroy any sporulated oocysts.

Therefore, we suggest to carrying out projects for the prevention of future problems with other animals and employees of the Bauru Municipal Zoo, as well as to promote health education activities regarding the main zoonotic diseases in the zoo environment, directed to zoo workers, so that the risk of toxoplasmosis as well as other zoonotic diseases can be prevented.

CONCLUSION

Our results showed the need for regular serological monitoring for toxoplasmosis in NHP, as well as analysis of the probable sources of infection, possibly related to the food and/or water provided to zoo animals and the presence of synanthropic animals in the enclosures, in addition to the probable reuse of sand from enclosures of other species, such as felines.

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DECLARATION OF CONFLIT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Muñoz R, Hidalgo-Hermoso E, Fredes F, Alegría-Morán R, Celis S, Ortiz-Tacci C, et al. Serological prevalence and risk factors of *Toxoplasma gondii* in Zoo Mammals in Chile. *Prev Vet Med.* 2021;194:105445.
2. Dubey JP, editor. *Toxoplasmosis of animals and humans.* 2nd ed. Boca Raton: CRC Press; 2010.
3. Paula NF, Dutra KS, Oliveira AR, Santos DO, Rocha CEV, Vitor RWA, et al. Host range and susceptibility to *Toxoplasma gondii* infection in captive Neotropical and Old-world primates. *J Med Primatol.* 2020;49(4):202-10.
4. Niehaus C, Spínola M, Su C, Rojas N, Rico-Chávez O, Ibarra-Cerdeña CN, et al. Environmental factors associated with *Toxoplasma gondii* Exposure in Neotropical Primates of Costa Rica. *Front Vet Sci.* 2020;7:583032.
5. Minervino AHH, Cassinelli ABM, Souza AJS, Alves MM, Soares MCP, Ferreira DAC, et al. Detection of *Toxoplasma gondii* antibodies in captive non-human primates in the Amazon region, Brazil. *J Med Primatol.* 2017;46(6):343-6.

6. Desmonts G, Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *J Clin Microbiol.* 1980;11(6):562-8.
7. Pimentel JS, Gennari SM, Dubey JP, Marvulo MF, Vasconcellos SA, Morais ZM, et al. Serological survey for toxoplasmosis and leptospirosis in neotropical wild mammals of the Aracaju Zoo, Sergipe. *Pesqui Vet Bras.* 2009;29(12):1009-14.
8. Pena HFJ, Marvulo MFV, Horta MC, Silva MA, Silva JCR, Siqueira DB, et al. Isolation and genetic characterisation of *Toxoplasma gondii* from a red-handed howler monkey (*Alouatta belzebul*), a jaguarundi (*Puma yagouaroundi*), and a black-eared opossum (*Didelphis aurita*) from Brazil. *Vet Parasitol.* 2011;175(3-4):377-81.
9. Alvarado-Esquivel C, Gayosso-Dominguez EA, Villena I, Dubey JP. Seroprevalence of *Toxoplasma gondii* infection in captive mammals in three zoos in Mexico City, Mexico. *J Zoo Wildl Med.* 2013;44(3):803-6.
10. Ferreira DRA, Ribeiro VO, Laroque PO, Wagner PGC, Pinheiro JW Jr, Silva JCA, et al. Risk factors associated with *Toxoplasma gondii* infection in captive *Sapajus* spp. *Am J Primatol.* 2015;77(5):558-62.
11. Smith JCR. Toxoplasmosis. In: Cubas ZS, Silva JCR, Catao-Dias JL, editors. *Wildlife treaty.* São Paulo: Roca; 2006. p. 768-84.
12. Silva RC, Langoni H, Su C, Silva AV. Genotypic characterization of *Toxoplasma gondii* in sheep from Brazilian slaughterhouses: new atypical genotypes and the clonal type II strain identified. *Vet Parasitol.* 2011;175(1-2):173-7.
13. Camargo ME. Fluorescent antibody test for the serodiagnosis of American trypanosomiasis. Technical modification employing preserved culture forms of *Trypanosoma cruzi* in a slide test. *Rev Inst Med Trop Sao Paulo.* 1966;8(5):227-34.
14. Homan W, Vercammen M, Braekeleer J, Verschueren H. Identification of a 200-to 300-fold repetitive 529bp DNA fragment in *Toxoplasma gondii* and its use for diagnostic and quantitative PCR. *Int J Parasitol.* 2000;30(1):69-75.
15. Mackinnon A. A spreadsheet for the calculation of comprehensive statistics for the assessment of diagnostic tests and inter-rater agreement. *Comput Biol Med.* 2000;30(3):127-34.
16. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Yang Y, Su C. Recent epidemiologic, clinical, and genetic diversity of *Toxoplasma gondii* infections in non-human primates. *Res Vet Sci.* 2021;136:631-41.
17. Grumann MR, Silva Z, Silva Filho JR, Costa MM, Vieira MIB, Motta AC. Immunohistochemical and serological aspects of *Toxoplasma gondii* infection in Neotropical primates. *Semina Cienc Agrar.* 2017;38(3):1375-82.

18. Silva RC, Machado GP, Cruvinel TMA, Cruvinel CA, Langoni H. Frequency of *Toxoplasma gondii* antibodies in tufted capuchin monkeys (*Cebus apella nigrurus*) from an ecological station in the State of São Paulo, Brazil. *Pesqui Vet Bras*. 2013;33(2):251-3.
19. Cano-Terriza D, Almería S, Caballero-Gómez J, Díaz-Cao JM, Jiménez-Ruiz S, Dubey JP, et al. Serological survey of *Toxoplasma gondii* in captive nonhuman primates in zoos in Spain. *Comp Immunol Microbiol Infect Dis*. 2019;65:54-7.
20. Akue JP, Tomo NE, Badiambile J, Moukana H, Mbou-Mountsimbi RA, Ngoubangoye B. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in non-human primates at a primate center at Franceville, Gabon. *J Parasitol Vector Biol*. 2018;10(1):1-7. Available from: <http://www.academicjournals.org/JPVB>. Accessed 21 Nov 2022.
21. Cola GA, Garcia JL, Costa L, Ruffolo B, Navarro IT, Freire RL. Comparação da reação de imunofluorescência indireta e do teste de aglutinação modificado na detecção de anticorpos anti-*Toxoplasma gondii* em ratos. *Semina Cienc Agrar*. 2010;31(3):717-22.
22. Villar-Echarte G, Arruda IF, Barbosa AS, Guzmán RG, Augusto AM, Troccoli F, et al. *Toxoplasma gondii* among captive wild mammals in zoos in Brazil and Cuba: seroprevalence and associated risk factors. *Braz J Vet Parasitol*. 2021;30(2):e001921.
23. Xin S, Jiang N, Yang L, Zhu N, Huang W, Li J, et al. Isolation, genotyping and virulence determination of a *Toxoplasma gondii* strain from non-human primate from China. *Transbound Emerg Dis*. 2022;69(2):919-25.

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