

Occurrence of *Toxoplasma gondii* antibodies in lowland tapirs maintained ex situ in Brazil and Paraguay

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ABSTRACT: Lowland Tapir (*Tapirus terrestris*) is the second largest South American land mammal. It is strictly herbivorous and its exposure to *Toxoplasma gondii* should be indicative of environmental contamination by oocysts. In the present study antibodies to *T. gondii* in 47 Brazilian tapirs maintained ex situ in 10 Brazilian and in one Paraguayan institution were sought in serum samples by the modified agglutination test (MAT ≥ 25). None of the animals presented clinical signs during the study. From 47 animals 35 (74.5%) were positive with titers of 25 in 8, 50 in 6, 100 in 12, 200 in 5, 400 in 1 and 800 in 3. One animal had samples collected on twice, and 19 were born in captivity. There was no association between occurrence of *T. gondii* antibodies and gender, and positive animals were reported in all institutions. The high occurrence of seropositive tapirs born in institutions (54.3%) confirmed the high exposure of these mammals to *T. gondii* in captivity. Only two cases of abortion were reported, but it was not possible to correlate these abortions to *T. gondii* infection.

Key words: Ex situ, lowland tapir; *Tapirus terrestris*, *Toxoplasma gondii*, seroprevalence.

Ocorrência de anticorpos para *Toxoplasma gondii* em antas brasileiras mantidas ex situ no Brasil e no Paraguai

RESUMO: A anta brasileira (*Tapirus terrestris*) é o segundo maior mamífero terrestre da América do Sul. É estritamente herbívora e sua exposição ao *Toxoplasma gondii* indica contaminação ambiental por oocistos. No presente estudo, 47 antas brasileiras mantidas ex-situ, em 10 instituições brasileiras e em uma paraguaia, tiveram amostras de soro avaliadas para a presença de anticorpos para *T. gondii* pelo Teste de Aglutinação Modificada (MAT ≥ 25). Dos 47 animais, 35 (74,5%) foram positivos, com títulos de 25 em 8, 50 em 6, 100 em 12, 200 em 5, 400 em 1 e 800 em 3 animais. Um animal teve amostras coletadas em duas ocasiões e, 19 nasceram em cativeiro. Não houve associação entre ocorrência de anticorpos para *T. gondii* e gênero e, animais positivos foram encontrados em todas as instituições. A alta ocorrência de antas soropositivas nascidas nas instituições (54,3%) confirma a alta exposição desses mamíferos para *T. gondii* em cativeiro. Apenas dois casos de aborto foram relatados, porém, não foi possível correlacioná-los à infecção pelo *T. gondii*.

Palavras-chave: Anta brasileira, Ex situ, *Tapirus terrestris*, *Toxoplasma gondii*, seroprevalência.

The protozoan parasite *Toxoplasma gondii* infects virtually all endothermic animals, including humans (DUBEY, 2010). Felids, both domestic and wild, are the only known definitive hosts, and the other endothermic animals are considered intermediate hosts and can become infected through ingestion of water or food contaminated by oocysts or by eating tissue cysts containing the parasite bradyzoites (DUBEY, 2010).

Tapirus terrestris, known as Brazilian tapir or lowland tapir, is the second largest South American land mammal (EISENBERG & REDFORD, 1999)

and has the widest distribution. *T. terrestris* is exclusively herbivorous, and is listed as vulnerable to extinction, according to the International Union for Conservation of Nature (FURTADO et al., 2010; NAVEDA et al., 2011).

High prevalence of anti-*T. gondii* antibodies have been detected in zoo animals around the world (SILVA et al, 2001; DE CAMPS et al 2008). Although few data on the infection by the parasite in tapirs is available, the modified agglutinating test (MAT) has already been used to identify antibodies

in captive and free-living individuals of this species (NAVEDA et al., 2011). In this study the presence of *T. gondii* antibodies was determined in *T. terrestris* kept ex situ in institutions located in Brazil and Paraguay.

Between November 2010 and December 2012, *T. terrestris* from 10 Brazilian and one Paraguayan institution had blood samples collected. In total, samples were obtained from 47 tapirs (18 adult females, one sub-adult female, one juvenile female, 19 adult males, three sub-adult males and five juvenile males). Individuals were separated in juvenile (6 months to 1 year), sub-adult (1-4 years), and adult (over 4 years of age) according known age or estimations via tooth wear and appearance of foot cushions and corporal measurements (MEDICI, 2010). One animal had blood samples collected on two occasions (November 2010 and October 2011). Eight institutions were located in the southeastern region of Brazil (São Paulo, Minas Gerais and Espírito Santo States) and two in the southern region of Brazil (Paraná State). The Paraguayan institution is located in the Alto Paraná region.

Blood samples (10-50mL) were collected using puncture of a saphenous or cephalic vein or in their carpal/tarsal derived, in the medial access. Serum was separated by centrifugation and kept at -20°C until analysis.

Sera were processed using MAT at cut-off 1:25 and those positive were diluted base two, until the final titer. Positive and negative controls, previously known, were adopted (FURTADO et al., 2010; MINERVINO et al., 2010). The association between gender, age and the presence of *T. gondii* antibodies was analyzed using the chi-square test with Yates correction, and $P < 0.05$ were considered significant. To assess the relation of age on the presence or absence of *T. gondii* antibodies, was applied a logistic regression on the data after transforming them in zeros and ones. For statistical purposes, the animal that had blood samples collected in two occasions, just the second collection was taken into consideration. All the analyses were performed on the R software version 3.3.1 (R CORE TEAM, 2016).

Antibodies against *T. gondii* (MAT \geq 25) were reported in 74.5% (35/47) tapirs (Table 1). The highest titer was 800, reported in three animals. Antibodies were detected in 75% of females (15/20) and 74.1% of males (20/27). No correlation was observed between gender and presence of *T. gondii* antibodies. Concerning age, 30 out of 37 adults (81.1%), two out of the four sub-adults (50%) and three out of the six juveniles animals (50%) showed

titer \geq 25. No relationship between age on presence or absence of *T. gondii* antibodies was reported ($P=0.152$). This reinforces the hypothesis that the simple exposure of animals to oocysts is a determinant factor of this contamination. *T. gondii* positive animals were reported in all assessed institutions. The animal that was assessed in two occasions was negative in the first collection (November 2010) and had a titer of 50 in the second (October 2011), indicating that infection occurred between collections.

Although the presence of antibodies against *T. gondii* had already been reported in tapirs (ZHANG et al., 2000; FURTADO et al., 2010; MINERVINO et al., 2010) this is the first study with a large sampling space and involving animals from different locations. The transmission of *T. gondii* in zoos is extremely important, because resistant and highly sensitive species to *T. gondii*, to which the disease can be fatal, are living in the same environment (DE CAMPS et al., 2008). All 47 assessed tapirs were reported in good health conditions on clinical examination, even the three animals that had the highest titer (800), suggesting that in this species the infection probably occurs asymptotically, as observed in most affected species (DUBEY & JONES, 2008).

Two female tapirs, with titers of 100 and 50, had given birth to healthy pups one and three months before tested, respectively. However, two other females, with titers of 25 and 800, had a recent miscarriage history. Although *T. gondii* is associated with congenital infection and abortion (DUBEY & JONES, 2008), there are no studies associating reproductive losses to *T. gondii* infection in this animal species. The MAT employed here detected IgG antibodies and seropositivity indicated exposure to the parasite. Although MAT has not been validated in tapirs, a MAT titer of 1:25 is considered as one of the most sensitive and specific tests for toxoplasmosis in animals (DUBEY & JONES, 2008).

Similar occurrence values were detected in females (75%) and male (74.1%) animals, comparable to observations also made in other studies with wild animals, what indicated that animals of both sexes are equally exposed to *T. gondii*. (SILVA et al., 2001; CARME et al., 2002; MINERVINO et al., 2010).

Nineteen out of 35 (54.3%) positive animals were born at the institution where the collection was performed, confirming that the infection occurred in this environment. It could reflect an error in the institutional management, facilitating the contact with oocysts by the environment, food, or water (ANDRÉ et al., 2010). It was not possible to determine the sources of infection but the high

Table 1 - Institutions, location and number of *Tapirus terrestris* sampled and positive to the presence of antibodies to *T. gondii*.

| Institution | Location City/State | N. positive/ N. examined (%) |
|-------------|----------------------------|------------------------------|
| 1 | Campinas/SP | 1/2 (50.0) |
| 2 | Sorocaba/SP | 2/6 (33.3) |
| 3 | Bauru/SP | 1/1 (100.0) |
| 4 | São Bernardo do Campo/SP | 2/2 (100.0) |
| 5 | Tapiraí/SP | 2/2 (100.0) |
| 6 | Marechal Floriano/ES | 2/4 (50.0) |
| 7 | Cachoeiro do Itapemirim/ES | 2/2 (100.0) |
| 8 | Poços de Caldas/MG | 1/1 (100.0) |
| 9 | Foz do Iguaçu/PR | 2/2 (100.0) |
| 10 | Foz do Iguaçu/PR | 4/6 (66.7) |
| 11 | Hernandarias/AP | 16/19 (84.2) |
| Total | | 35/47 (74.5) |

exposure in tapirs is probably associated with the ingestion of oocysts present in the feed, water or pasture provided, since tapirs are strict herbivores and diet in captivity is usually based on horse feed, forage, grains, fruits and vegetables (CLAUSS et al., 2009). Most of these foods provided and pasture can easily be contaminated by feral cat feces or by mechanical transportation of oocysts shed by captive wild felids and passed through keeper's clothing, boots, cleaning equipment or by transport hosts as birds and insects (ANDRÉ et al., 2010). So it is very important acquire and store feed carefully, as control the existence of feral cats in the zoo vicinities, preventing food and environmental contaminations (DE CAMPS et al., 2008). *T. gondii* oocysts are highly resistant, and to the routine, there is no easy and convenient method to kill them on fruits and vegetables, other than cooking until the temperature reaches 60°C (DE CAMPS et al., 2008).

The authors would like to thank the Foundation for Research Support of the Espírito Santo (FAPES - Processes 50239368 and 54684137), and the staff of all the sampled Institutions for their support during collection. We also thank Drs. A. Marcili, L. F. Tobias, P. R. Mangini, R. C. Santos, and E. R. Monteiro for their support in the development of this research.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All procedures were performed under the license number 34372-1, from the Brazilian Institute of Environment and

Renewable Natural Resources (IBAMA) and the approval number 175-2011 from the Committee of Ethics in the Use of Animals of Universidade de Vila Velha (CEUA-UVV)

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