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Rabies Virus Exposure in Wild Lowland Tapirs (*Tapirus terrestris*) from Three Brazilian Biomes

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ABSTRACT: We evaluated the presence of antibodies for rabies virus in 177 serum samples from 125 wild lowland tapirs (*Tapirus terrestris*) from three different Brazilian biomes. The rapid fluorescent focus inhibition test was performed. No antibody titers suggesting the circulation of the rabies virus in tapir habitat were detected.

Rabies is an acute and highly lethal viral disease to which all warm-blooded animals are susceptible, including humans (Megid 2014). Transmission occurs mainly through saliva, and chiropteran and carnivoran species are considered the most common hosts of the virus (WHO 2018). Knowledge about the participation of other wild hosts in the epidemiologic cycle of the disease is still incipient, making control and prophylaxis of rabies challenging (Almeida et al. 2001; Jorge et al. 2010; Araújo et al. 2014; Megid 2014). In herbivores, rabies occurs mainly in cattle (*Bos taurus*) and may be responsible for considerable economic losses (Oviedo-Pastrana et al. 2015).

Our study focused on the lowland tapir (*Tapirus terrestris*), a large wild herbivore of the order Perissodactyla, which is considered a low-susceptibility group to rabies virus (Megid 2014). Nevertheless, the Chiroptera-related virus may be a source of rabies infection in tapirs. Hematophagous bats (*Desmodus rotundus*) feed on lowland tapirs in the wild (Galetti et al. 2016; Gnocchi and Srbek-Araujo 2017). According to Galetti et al. (2016), there is an 11% chance of a tapir being bitten by a bat in the Pantanal region of Brazil, and a 0.15% probability for rabies transfer to tapirs by bats in the same biome. Additionally, antibody titers representing exposure to the virus have been recently described in a blood sample collected from a tapir carcass in São Paulo state, Brazil (Antunes et al. 2017). However, the authors neither specified how long after death the sample was collected nor discussed the diagnostic value of antibody detection in blood samples from postmortem screenings.

Our goal was to evaluate the presence of neutralizing antibodies specific for rabies virus in tapir serum samples from the Brazilian Atlantic Forest, Pantanal, and Cerrado biomes collected between 2006 and 2018. Our study site in the Atlantic Forest biome was Morro do Diabo State Park, located in western São Paulo state (22°16′S, 52°05′W). In the Pantanal, the study area was a private cattle ranch located in Mato Grosso do Sul state (19°20′S, 55°43′W). The Cerrado sampling site was a mosaic of different types of land use on both sides of an important highway of Mato Grosso do Sul state (21°60′S, 53°83′W).

In Brazil, rabies is a compulsorily notifiable disease, and governmental agencies maintain a database of officially reported cases in each state of the Brazilian territory (Brasil Ministério da Agricultura 2020; Brasil Ministério da Saúde 2020). Table 1 shows rabies cases reported in humans, domestic animals, and wildlife in the states where our study was developed in the same sampling period. Several cases in domestic herbivores were reported, but scientific information about wild herbivores such as tapirs is lacking in rabies epidemiology. Our hypothesis was driven by the proximity of tapirs to domestic livestock in our study sites, along with bat feeding behavior and the ability of rabies virus to spill

TABLE 1. Rabies cases reported in humans, domestic animals, and wildlife in São Paulo state (2006–08) and Mato Grosso do Sul state (2009–18), Brazil, in the same period of our survey (Brasil Ministério da Agricultura 2020; Brasil Ministério da Saúde 2020). This table shows confirmed cases within the territory of each state, and not specifically inside our study areas.

Rabies cases[a]	São Paulo state	Mato Grosso do Sul state
Sampling period	2006–08	2009–18
Human	0	1
Bovine	258	1,213
Equine	48	91
Other domestic herbivore	1	1
Canine	0	86
Wildlife[b]	151	35
Total	458	1,427

[a] Individuals sick or infected with confirmed diagnosis.

[b] The data do not specify, but the vast majority of wild animals tested were hematophagous and nonhematophagous bats.

over, which creates opportunities for disease spreading and may represent a serious threat for human, domestic animal, and wildlife health.

Capture methods and anesthetic protocols were applied as described (Medici et al. 2014; Fernandes-Santos et al. 2020). About 60 mL of blood was collected within 20 min of immobilization through venipuncture of the medial saphenous or cephalic veins with vacuum sampling tubes. Samples were placed in a portable cooler with ice and immediately transported to a field laboratory. Blood samples without anticoagulant were centrifuged at 2000 × G for 15 min within 12 h of collection, and serum aliquots were frozen at –26 C in a biobank in Campo Grande, Mato Grosso do Sul, Brazil. Serologic tests were carried out in a reference laboratory in Brazil (Pasteur Institute, São Paulo, São Paulo, Brazil).

Rapid fluorescent focus inhibition test was performed according to Rupprecht et al. (2018) with modifications. We used the standard international serum (WHO 2018), and samples were diluted into six serial fivefold dilutions starting from 1:2.5 in 96-well microplates. After the challenge virus standard suspension (CVS-132-11A) addition and incubation, a BHK-21 cell suspension (2.5×10^5 cells/mL) were added, and microplates were incubated again. Plate cells were fixed with acetone and incubated with rabies virus antibodies in isothiocyanate-conjugated fluorescein, and Chimeric antigen receptor–immunoprecipitation conjugate produced at the serology laboratory of the Pasteur Institute (Medeiros Caporale et al. 2009). The reading was performed under a 200-fold inverted fluorescence microscope, and the total area of each plate hole was examined. Titers were calculated by the Spearman-Kärber analysis method compared with the standard serum.

We evaluated 125 different individuals plus 52 recaptures, totalizing 177 serum samples. Our results showed titers of neutralizing antirabies antibodies ranging between <0.01 and 0.07 IU/mL (Table 2).

Antibody titers ≥0.5 IU/mL are considered protective or indicate viral exposure (Almeida et al. 2001; WHO 2018). Some studies with wildlife species have considered those animals with neutralizing antibody titers ≥0.1 IU/mL positive (Jorge et al. 2010; Antunes et al. 2017). No antibody titers against rabies virus that might suggest circulation of the infectious agent in their habitat were found in wild lowland tapirs evaluated in this study.

Serology is an important surveillance tool. However, rapid fluorescent focus inhibition test results should be carefully interpreted. Negative findings are inconclusive because neutralizing antibody often is not present until clinical stages of the disease, and titer levels <0.1 IU/mL may be associated with cross-reactive antibodies (Rupprecht et al. 2018).

Rabies is a fatal disease, and affected mammals may die shortly after infection, which emphasizes the relevance of monitoring individuals in wildlife research. Tapirs assessed have been monitored over the long term, and only 11 deaths have been reported in these populations—four by predation, three by collision with vehicles, and four by unknown causes (Medici et al. 2014; Fernandes-Santos et al. 2020). Several bat feeding events on tapirs, cattle, and feral pigs (*Sus*

TABLE 2. Sampling description and titers of neutralizing anti-rabies antibodies detected in wild lowland tapirs (*Tapirus terrestris*) from three different Brazilian biomes (2006–18).

	Atlantic Forest[a]	Pantanal[b]	Cerrado[b]	Total
Sampling period	2006–08	2009–18	2015–17	2006–18
Sample size[c]	10	129	38	177
Individuals[d]	10	80	35	125
Sex ratio (M:F)[e]	4:6	49:31	14:21	67:58
Age classes (A:SA:J:C)[f]	7:1:2:0	78:39:9:3	26:8:4:0	111:48:15:3
Antibody titers (IU/mL)	<0.02–0.03	<0.01–0.06	<0.01–0.07	<0.01–0.07

[a] São Paulo state, Brazil.
[b] Mato Grosso do Sul state, Brazil.
[c] Including captures and recaptures.
[d] Number of different individuals sampled in each biome.
[e] M = male; F = female.
[f] A = adult; SA = subadult; J = juvenile; C = calf.

scrofa) have been recorded by camera traps in our Pantanal study site. Despite our results, the potential for rabies virus to spill over between domestic, feral, and wild animals in the same habitat represents an important threat to be considered, and monitoring wild populations is critical for rabies surveillance.

The study of tapir health has been an important component of the long-term activities of the Lowland Tapir Conservation Initiative (LTCI)—Instituto de Pesquisas Ecológicas in Brazil. The Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis and Instituto Chico Mendes de Conservação da Biodiversidade provided the required annual permits for the capture and immobilization of tapirs and collection of biological samples (SISBIO 52324269). All protocols for the capture, immobilization, manipulation, and sampling of tapirs have been reviewed and approved by the Veterinary Advisors of the Association of Zoos and Aquariums Tapir Taxon Advisory Group and the Veterinary Committee of the International Union for Conservation of Nature Species Survival Commission (IUCN SSC) Tapir Specialist Group. The LTCI has institutional support from the IUCN SSC Tapir Specialist Group, Association of Zoos and Aquariums Tapir Taxon Advisory Group, and European Association of Zoos and Aquariums Tapir Taxon Advisory Group. Over the years, the LTCI has been funded by several national and international agencies, including >50 zoological institutions, 22 nongovernmental organizations and foundations, and 11 corporations, as well as private individuals. The LTCI is deeply grateful to the Pasteur Institute for carrying out the analysis for rabies. The authors thank Caroline Testa José and Ariel Canena for their dedication in data collection and Alexandra Tiso Cumerlato and Ricardo Corassa Arrais for their revisions of previous versions of this article.

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