No evidence of SARS-CoV-2 infection in Neotropical Primates sampled during COVID-19 pandemic in Minas Gerais and Rio Grande do Sul, Brazil.

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Abstract

In 2019, a new coronavirus disease (COVID-19) was detected in China. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was capable to infect domestic and captive mammals like cats, tigers and minks. Due to genetic similarities, concern about the infection of Non-Human Primates (NHPs) and the establishment of a sylvatic cycle has grown in the Americas. In this study, neotropical primates (NP) were sampled in different areas from Brazil to investigate whether they were infected by SARS-CoV-2. A total of 89 samples from 51 NP of four species were examined. No positive samples were detected via RT-qPCR, regardless of the NHP species, tissue or habitat tested. This work provides the first report on the lack of evidence of circulation of SARS-CoV-2 in NP. The expand of wild animals sampling is necessary to understand their role in the epidemiology of SARS-CoV-2.

Key words: *Coronavirus*; *Callithrix*; *Alouatta*; Spillover; Spillback; Nonhuman-primate

In December 2019, a new disease caused by a virus belonging to *Coronaviridae* family, was first detected in the city of Wuhan, China, and was named coronavirus disease (COVID-19) (Wu et al. 2020). The etiologic agent - Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) - is highly transmissible among humans through droplets of saliva and quickly spread across the planet. In March 2020, the virus reached every continent which led the World Health Organization to declare COVID-19 as a pandemic (World Health Organization).

Initial studies of epidemiology and genetic comparison showed that the new virus has high similarity to other coronaviruses found in bats and pangolin (96 and 91% similarity, respectively) (Zhang et al. 2020) and that the “spillover”, that is, the “jump” between hosts could have occurred in the vicinity of the popular market in Wuhan, where these animals are traditionally traded for human consumption (Wu et al. 2020). Thenceforth, it has been questioned whether SARS-CoV-2 would be able to perform a “spillback” - transmission from humans to wild animals -, which could generate wild cycles of the virus, making it even more difficult to face.

In this regard, concern about the infection of non-human primates (NHPs) has grown since the biological, genetic and biochemical similarity, could facilitate the transmission of SARS-CoV-2 to NHPs. Comparative studies have indicated that the cellular receptor angiotensin-converting enzyme 2 (ACE2), used by this virus for adsorption to cells, is similar between humans and NHPs, especially in Old World species; as such, these have been used as experimental models for the study of SARS-CoV-2 (Melin et al. 2020). In the Americas, there is a great concern about the risks of SARS-CoV-2 spillback from humans to wildlife, including neotropical NHPs. This could have at least two impacts: a) establishment of a sylvatic cycle with serious implications on control or eradication efforts, and: b) unforeseeable impact on biodiversity, especially if endangered NHP species become involved. The historical comparison with yellow fever virus (YFV), whose sylvatic cycle was first recognized almost a century ago after a spillback from human to NHPs, exemplifies the potential damage that may be associated to such impacts (Possas et al. 2018).

To our knowledge, the only neotropical primate species experimentally infected with SARS-CoV-2 was the marmoset *Callithrix jacchus*. Infected individuals had pyrexia and viral RNA loads detectable for several days post-infection in oral, nasal and anal swabs, as well as in blood and stool samples (Lu et al. 2020). However, little is known about the possibility of viral infection in natural conditions and in other neotropical primates' families or species. Worryingly, in many Brazilian cities where cases of COVID-19 are currently reaching unprecedented levels, *Callithrix* spp. are adapted to synanthropic environments, often kept as pets and feeding on human foods. Such coexistence might represent a significant potential source of infection (Longa et al. 2011). In addition, the rapid dissemination of SARS-CoV-2 currently verified throughout Brazil can lead to the exposure of other NHPs, including wild species.

The present study was carried out in search for serological and virological evidences of SARS-CoV-2 infections in NHPs from urban, sylvatic and rural areas of two Brazilian states, Minas Gerais and Rio Grande do Sul. The current study is part of a wider NHP sampling effort whose main goal is to contribute to the national program for epidemiological surveillance of yellow fever virus.

Neotropical Primates' samplings were performed before (November 2019) and during (July 2020 - February 2021) the COVID-19 pandemic in two Brazilian states. First in Minas Gerais (Southeast region), where the savannah-like biome predominates, captures occurred in four (Fig. 1A). After in Rio Grande do Sul (South region), covered by Atlantic Forest and Pampa biomes, in seven municipalities (Fig. 1B). Human COVID-19 cases per 100.000 inhabitants up to the sampling efforts are highlighted by the colors of the municipalities on the map (SES-MG 2020; SES-RS 2021).

Live marmosets were captured with Tomahawk traps, according to the protocol previously described (Abreu et al. 2019a). Dead or sick marmosets and howler monkeys were also evaluated. In MG, they were found based on an information network made up of health professionals, farmers, and other people in contact with natural environments, as described elsewhere (Abreu et al. 2019b) while in RS, Health Department network people found and informed the epizooties. Serum and/or oral swab, and viscera (lung, liver, spleen or kidney of dead animals) were collected and preserved in RNA*later* (Thermo Fisher™) and stored in liquid nitrogen at -186ºC. Our methods and protocols were previously approved by the Institutional Ethics Committee for Animal Experimentation (Protocol CEUA/IFNMG nº14/2019) and by Brazilian Ministry of the Environment (SISBIO nº 71714-2).

RNA extractions were performed using QIAamp Viral RNA Mini Kit (QIAGEN® (for serum) and TRIzol™ Reagent (Thermo Fisher™) (for viscera) following manufacturer's instructions. Detection of the SARS-CoV-2 viral RNA in samples from Minas Gerais was performed using a real-time RT-PCR kit (CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, which targets the N1 (2019-nCoV_N1 Combined Primer/Probe Mix) and N2 (2019-nCoV_N2 Combined Primer/Probe Mix) genes of the SARS-CoV-2 (CDC Division of Viral Diseases 2020). In Rio Grande do Sul, detection was performed using the Allplex™ 2019-nCoV (Seegene®), which targets genomic regions within the *E*, *RdRP*, and *N* genes.

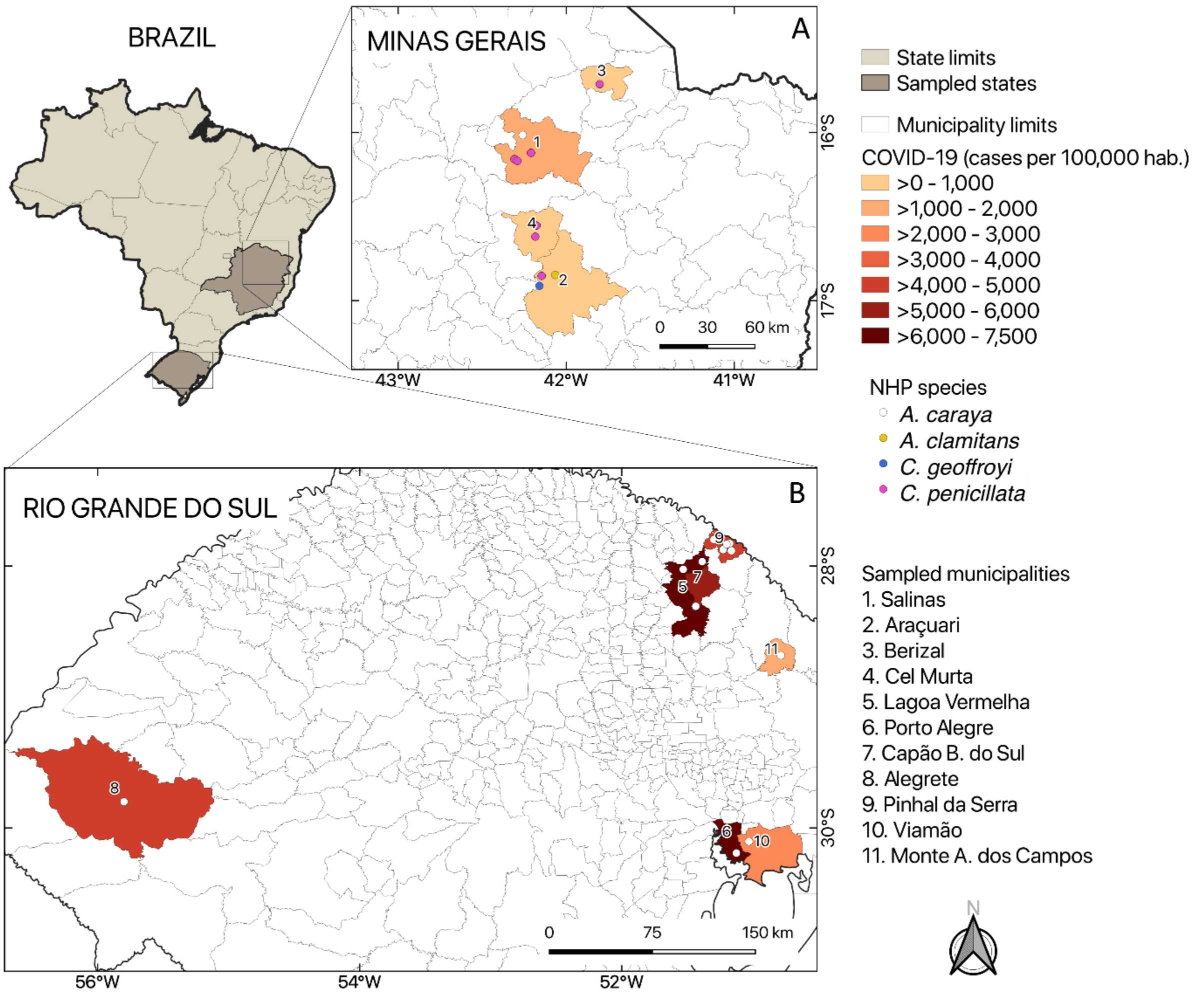

Figure 1: Municipalities where sampling of NHPs was performed. A: Minas Gerais state; B: Rio Grande do Sul state. The number of COVID-19 human cases in each municipality were counted until the sample collection date. Data were obtained from state health departments (SES-MG 2020; SES-RS 2021). The figure was done using QGIS software, version 3.10.

In total, 51 NHPs belonging to two different families (Callitrichidae and Atelidae) and four species (*Callithrix penicillata*, *C. jacchus*, *Alouatta caraya* and *A. guariba clamitans*) were examined (Table 1). Seven NHPs were captured before the reported virus introduction (august - november / 2019) and 44 after the introduction (july 2020 to february 2021). Different tissues were analyzed, totalizing 89 samples: 30 of serum, 20 of oral swabs, 19 of liver, 14 of kidney, 3 of lung and 3 of whole blood (Table 1). No positive samples were detected, regardless of NHP species, tissue, habitat, municipalities or state/biome tested (Table 1).

Table 1: Description of samples tested by species, tissue, habitat, date, municipality (city) and state.

ID	Species	Serum	Oral swab	Whole blood	Liver	Lung	Kidney	Habitat	Collection date	City	State
MG02	*A. caraya*	_	_	Neg	Neg	Neg	_	Sylvatic	20/08/2019	1	MG
MG03	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG04	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG05	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG06	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG07	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG08	*C. penicillata*	Neg	_	_	_	_	_	Rural	22/11/2019		
MG10	*C. penicillata*	Neg	_	_	_	_	_	Sylvatic	04/07/2020		
MG11	*C. penicillata*	Neg	_	_	_	_	_	Sylvatic	04/07/2020		
MG12	*C. penicillata*	Neg	_	_	_	_	_	Sylvatic	04/07/2020		
MG13	*C. penicillata*	Neg	_	_	_	_	_	Sylvatic	04/07/2020		
MG21	*C. penicillata*	_	Neg	_	Neg	_	_	Urban	30/08/2020		
MG22	*C. penicillata*	_	Neg	Neg	Neg	_	_	Urban	30/08/2020		
MG23	*C. penicillata*	_	Neg	Neg	Neg	_	_	Urban	30/08/2020		
MG24	*C. penicillata*	Neg	Neg	_	_	_	_	Urban	12/09/2020		
MG25	*C. penicillata*	Neg	Neg	_	_	_	_	Urban	19/09/2020		
MG26	*C. penicillata*	Neg	Neg	_	_	_	_	Urban	21/09/2020		
MG14	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020	2	
MG15	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020		
MG17	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020		
MG18	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020		
MG19	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020		
MG20	*C. penicillata*	Neg	_	_	_	_	_	Rural	30/07/2020		

MG28	*C. penicillata*	_	Neg	_	_	_	_	Rural	18/10/2020	3	
MG29	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	18/10/2020		
MG30	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	18/10/2020		
MG31	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	18/10/2020		
MG32	*C. geoffroyi*	Neg	Neg	_	_	_	_	Rural	18/10/2020		
MG33	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	19/10/2020	4	
MG34	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	19/10/2020		
MG35	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	20/10/2020		
MG36	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	20/10/2020		
MG37	*C. penicillata*	_	Neg	_	_	Neg	_	Rural	20/10/2020		
MG38	*C. penicillata*	Neg	Neg	_	_	_	_	Rural	20/10/2020		
MG39	*C. penicillata*	Neg	Neg	_	_	_	_	Urban	21/10/2020		
MG40	*C. penicillata*	Neg	Neg	_	_	_	_	Urban	21/10/2020		
MG41	*A. clamitans*	_	Neg	_	Neg	Neg	_	Urban	22/10/2020		
RS01	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	25/01/2021	5	RS
RS02	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	25/01/2021		
RS03	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	25/01/2021		
RS04	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	5/2/2021		
RS05	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	8/2/2021		
RS06	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	9/2/2021		
RS07	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	11/2/2021		
RS08	*A. caraya*	_	_	_	Neg	_	Neg	Urban	11/2/2021	6	
RS09	*A. clamitans*	_	_	_	Neg	_	Neg	Rural	16/2/2021	7	
RS10	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	10/2/2021	8	
RS11	*A. clamitans*	_	_	_	Neg	_	Neg	Urban	15/1/2021	9	
RS12	*A. clamitans*	_	_	_	Neg	_	Neg	Rural	2/2/2021	10	
RS13	*A. clamitans*	_	_	_	Neg	_	Neg	Sylvatic	5/2/2021		
RS14	*A. clamitans*	_	_	_	Neg	_	Neg	Rural	8/2/2021	11	

Legend: Neg: negative sample; - not tested; 1: Salinas; 2: Berizal; 3: Araçuaí; 4: Coronel Murta; 5: Pinhal da Serra; 6: Alegrete; 7: Porto Alegre; 8: Capão Bonito do Sul; 9: Viamão; 10: Lagoa Vermelha;11: Monte Alegre dos Campos. MG: Minas Gerais; RS: Rio Grande do Sul.

This is the first study evaluating the natural infection of free-living neotropical NHPs with SARS-CoV-2. We found no evidence of SARS-CoV-2 infection in NHPs from urban, rural or sylvatic habitats regardless of the tissue or species surveyed. Even so, it is important to maintain and expand the surveillance of wildlife, aiming at the early detection of spillover / spillback among human beings and other animals, considering the widespread of SARS-CoV-2 in Brazil.

Since the emergence, reports of natural SARS-CoV-2 infection of several animals have accumulated, including domestic animals like cats (Sailleau et al. 2020; Carlos et al. 2021) and wild captive animals like tigers (McAloose et al. 2020) and minks (Hammer et al. 2021). Minks have also been described as a source of infection for humans (Hammer et al. 2021). In addition, experimental infection studies have reported susceptibility of several animals such as ferrets, bat, hamsters (Shi et al. 2020; Imai et al. 2020), and mainly Old World NHPs (Zheng et al. 2020; Lu et al. 2020). As a result, concern about the SARS-CoV-2 spillback for Neotropical Primates – as happened with YFV in the last century and probably with ZIKA in the last decade – has grown (Terzian et al. 2018; Yunes Guimarães et al. 2020).

The only report of experimental infection in a neotropical NHP published to date, examined the dynamics of SARS-CoV-2 infection in *Callithrix jacchus*, the common marmoset. Viral genomes were detected in oral, nasal and anal swabs as well as in serum and feces, despite the lower susceptibility of the species when compared to Old World Primates. (Lu et al. 2020). In the present study, all tissues sampled from the 44 free-living primates collected after the introduction of the virus in Brazil were negative, including samples from NHP living in rural and urban environments, in close contact with humans.

The cellular receptor ACE2 used by the virus for adsorption to cells, is a major determinant in the susceptibility of animals to SARS-CoV-2 infection. The ACE2 receptor of cells from Old World NHPs have up to 99% identity with human ACE2 protein, what might contribute significantly to the high susceptibility that such species display to infection with this virus (Shi et al. 2020; Melin et al. 2020). Neotropical NHPs, such as those sampled in the present study, have ACE2 receptors that share about 92% identity to human ACE2, with 4 differences across the twelve binding sites regarded as essential for the ACE2 protein to allow binding of SARS-CoV-2 (Melin et al. 2020; Joyraj Bhattacharjee et al. 2021). This may be related to species' differences in susceptibility and, possibly, with our negative results, even in NHPs living in urban areas with high SARS-CoV-2 human incidence. Even so, it is important to investigate whether the new variants of SARS-CoV-2 could increase their infectivity in wild animals.

Brazil is the country with the greatest biodiversity on the planet and concentrates the largest number of NHP species, many of these endangered due to anthropogenic effects such as deforestation which, in addition to drastically reducing habitats, increases the risks of zoonotic transmissions (Paglia et al. 2012; Guthid et al. 2020). The explosive spread of SARS-CoV-2 in the country and the close contact between humans and native fauna, provides conditions for spillovers and spillback which increases the need for surveillance for early identification and management of potential harmful effects (Gryseels et al. 2020). In this sense and despite the small sampling, our study contributes to shedding light on the subject by providing the first results about the absence of circulation of SARS-CoV-2 in Neotropical Primates. It is urgent to expand the sampling of NHP living in different environments, to include other primate species and even other mammals to carry out an active surveillance of wild animals and understand the epidemiology of SARS-CoV-2 in natural environments.

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Conflict of interests /competing interests

The authors declare no conflict of interests or competing interests.

Ethics approval

All applicable institutional and/or national guidelines for the care and use of animals were followed. Methods and protocols were previously approved by the Institutional Ethics Committee for Animal Experimentation (Protocol CEUA/IFNMG nº14/2019) and by Brazilian Ministry of the Environment (SISBIO nº 71714-2). This article does not contain any studies with human participants.

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