

# Paracoccidioidomycosis in wild monkeys from Paraná State, Brazil

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**Abstract** The aim of this study was to evaluate the seroprevalence of *Paracoccidioides brasiliensis* infection in wild New World monkeys (*Cebus* sp. and *Alouatta caraya*). A total of 93 animals (*Cebus* sp.,  $n = 68$  and *Alouatta caraya*,  $n = 25$ ) were captured in the Paraná River basin, Paraná State, Brazil and the serum samples were analyzed by ELISA and immunodiffusion using *P. brasiliensis*

gp43 and exoantigen as antigens, respectively. The seropositivity observed by ELISA was 44.1% and 60% for *Cebus* sp. and *A. caraya*, respectively, while by immunodiffusion test *Cebus* sp. showed positivity of 2.9% only. No significant difference was observed in relation to age and sex. This is the first report of paracoccidioidomycosis in wild capuchin monkeys and in wild-black and golden-howler monkeys. The high positivity to *P. brasiliensis* infection in both species evaluated in our study and the positivity by immunodiffusion test in *Cebus* sp. suggest that natural disease may be occurring in wild monkeys living in paracoccidioidomycosis endemic areas.

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## Introduction

Paracoccidioidomycosis (PCM) is a systemic mycosis prevalent in Latin America and was described by Lutz in 1908 [1]. In Brazil, PCM is the eighth cause of death among infectious and parasite diseases [2].

The etiologic agent, *Paracoccidioides brasiliensis*, grows as mycelia when cultured at 25°C or as yeast when cultured at 37°C or in the host. The infection may occur by inhalation of fungus propagules and male agricultural workers are the most affected ones by the disease [3, 4]. The PCM can be classified as PCM infection (infected individuals living in PCM

endemic areas without symptoms of disease) and PCM disease (patients with PCM clinical symptoms) [5].

The role of other animal species in the fungus biological cycle is little understood. The infection of different species by *P. brasiliensis* has been evaluated by skin test or serological tests in domestic animals, such as dogs [6, 7], horses [8], and cows [9], as well as in captive wild animals, such as coati-mundi, felines, and capuchin monkeys [10]. The fungus was isolated several times from armadillos in Brazil [11–13] and Colombia [14] and the first two cases of natural PCM disease in dogs were reported recently [15, 16].

Taking into account the lack of data about eco-epidemiology of PCM the aim of this study was to evaluate *P. brasiliensis* infection in wild *Cebus* sp. (capuchin monkeys) and *Alouatta caraya* (black and golden howler monkeys).

## Materials & methods

### Area of study and animals

The municipality of Porto Rico (latitude 22°46'40" S, longitude 53°15'40" W, altitude 470 m) is located in Paraná State, Southern Brazil (Fig. 1). The climate is humid subtropical with temperatures ranging from 16 to 29°C. The animals were captured on islands and in forest reserves from the Paraná River basin. These



**Fig. 1** Map showing the municipality of Porto Rico in Paraná State

areas, mainly covered by sub-tropical forests, are environmentally protected by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA). The monkeys, apparently healthy, with strictly forest habits, (*Cebus* sp.,  $n = 68$  and *A. caraya*,  $n = 25$ ), were trap captured by veterinarians and biologists, who had a license from IBAMA (number 140/04). The blood samples were collected by venous puncture and the serum samples were stored at  $-20^{\circ}\text{C}$ .

### *P. brasiliensis* antigens

**Exoantigen:** The exoantigen was obtained as described by Camargo et al. [17], using the *P. brasiliensis* isolate B-339.

**gp43 antigen:** The gp43 antigen was purified from the *P. brasiliensis* exoantigen by affinity chromatography according to Puccia and Travassos [18] and the protein concentration was determined by the Bradford method using BSA as standard [19].

### ELISA with gp43 antigen

Polystyrene flat-bottom microtiter plates (Corning Costar Corporation, Corning, NY, USA) were coated with 100  $\mu\text{l}$  of gp43 in 0.1 M carbonate buffer, pH 9.6 (250 ng well $^{-1}$ ), for 18 h at 4°C. The plates were washed thrice with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and the wells were blocked with PBS-T 5% skim milk (PBS-T-M) for 1 h at 37°C. After washing thrice with PBS-T, the serum samples were diluted 1:100 in PBS 1% skim milk (PBS-M) and incubated at 25°C for 1 h. The plates were washed as above and 100  $\mu\text{l}$  of conjugate protein-A-peroxidase (Biorad, Hercules, CA, USA) were added to each well. Plates were then incubated at 37°C for 1 h. After washing thrice with PBS-T, 100  $\mu\text{l}$  of substrate/chromogen ( $\text{H}_2\text{O}_2$ /tetramethylbenzidine) were added to each well, and the reaction was stopped by adding 50  $\mu\text{l}$  of 4N  $\text{H}_2\text{SO}_4$ . Absorbance was measured with a Microplate Reader (Biotek Instruments Inc., Winooski, VT, USA) at 450 nm. Serum samples with two and half times the absorbance of the well without serum were considered positive.

## Immunodiffusion test

The immunodiffusion test was carried out as described previously [20], using *P. brasiliensis* exo-antigen as reagent. The serum samples were added at peripheral orifices and the exoantigen at the central orifice.

## Statistical analysis

The data were analyzed by the qui-square test and Fisher exact test (Epi Info Version 6.04b). The difference was considered significant when *P* was less than 0.05.

## Results and discussion

Several attempts to evaluate the infection of different domestic and wild animal species by *P. brasiliensis* have been made in order to clarify PCM eco-epidemiology.

The ELISA analysis of 68 serum samples from wild capuchin monkeys and 25 from black and golden howler monkeys showed positivity to *P. brasiliensis* antigen of 44.1% and 60%, respectively (Tables 1 and 2).

This is the first seroepidemiological study of PCM in wild monkeys. Two other studies of PCM in simians were carried out with captive animals using a skin test with polysaccharide antigen and showed positivity of 33.3% ( $n = 33$ ) and 21.1% ( $n = 52$ ) [21,

**Table 1** Reactivity to *P. brasiliensis* gp43 antigen, evaluated by ELISA and immunodiffusion test (ID), in serum samples from *Cebus* sp., according to sex and age

	Positive <i>n</i> (%)		Negative <i>n</i> (%)		Total <i>n</i> (%)
	ELISA	ID	ELISA	ID	
<i>Sex</i>					
Male	21 (46.7)	2 (4.4)	24 (53.3)	43 (95.6)	45 (66.2)
Female	9 (39.1)	0 (0)	14 (60.9)	23 (100)	23 (33.8)
<i>Age</i>					
Juvenile	9 (36.0)	1 (4.0)	16 (64.0)	24 (96.0)	25 (36.8)
Sub-adult	2 (66.7)	0 (0)	1 (33.3)	3 (100)	3 (4.4)
Adult	19 (47.5)	1 (2.5)	21 (52.5)	39 (97.5)	40 (58.8)
Total	30 (44.1)	2 (2.9)	38 (55.9)	67 (97.1)	68 (100)

**Table 2** Reactivity to *P. brasiliensis* gp43 antigen, evaluated by ELISA and immunodiffusion test (ID), in serum samples from *A. caraya*, according to sex and age

	Positive <i>n</i> (%)		Negative <i>n</i> (%)		Total <i>n</i> (%)
	ELISA	ID	ELISA	ID	
<i>Sex</i>					
Male	9 (64.3)	0 (0)	5 (35.7)	14 (100)	14 (56.0)
Female	6 (54.5)	0 (0)	5 (45.5)	11 (100)	11 (44.0)
<i>Age</i>					
Juvenile	2 (33.3)	0 (0)	4 (66.7)	6 (100)	6 (24.0)
Sub-adult	2 (66.7)	0 (0)	1 (33.3)	3 (100)	3 (12.0)
Adult	11 (68.8)	0 (0)	5 (31.2)	16 (100)	16 (64.0)
Total	15 (60.0)	0 (0)	10 (40.0)	25 (100)	25 (100)

22]. The lower positivity that was observed in these studies may be due to the fact that the animals were not in their natural habitat and consequently with lower risk of infection. On the other hand, in our study, the animals were captured in forests and on islands in the Paraná River basin, thus with frequent contact with the probable fungus habitat. Another explanation could be the difference between sensitivities of ELISA and skin tests.

The *P. brasiliensis* infection of male and females was not significantly different for *Cebus* sp. and *A. caraya*, suggesting that animals from both sexes are equally exposed to the fungus infection as observed previously in a seroepidemiological study of PCM in dogs [7].

The positivity to *P. brasiliensis* was not different in relation to age or to species (Tables 1 and 2), although a tendency to higher positivity was observed in the black and golden howler monkeys (Table 2). The habit of earth eating that was observed in howler monkeys [23] may contribute to *P. brasiliensis* infection taking into account that soil is the probable fungus habitat [24].

Jonhson and Lang [25] reported a case of natural PCM disease in a squirrel monkey (*Saimiri sciureus*) from Bolivia, indicating that non-human primates could be susceptible to PCM disease development. The positivity observed by immunodiffusion test in two serum samples from *Cebus* sp. reinforces this hypothesis (Table 1).

Taking into account the high positivity to *P. brasiliensis* infection in both species evaluated in our study it is possible that natural disease may be

occurring in wild monkeys living in PCM endemic areas.

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