

NEW RECORDS OF *HISTOPLASMA CAPSULATUM* FROM WILD ANIMALS IN THE BRAZILIAN AMAZON

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SUMMARY

Twenty-eight isolates of *Histoplasma capsulatum* were obtained from eight species of forest mammals from the States of Amazonas, Pará and Rondônia in the Amazon Region of Brazil. Primary isolates were obtained by inoculating triturated liver and spleen tissue intradermally and intraperitoneally in hamsters. Mycological diagnosis in hamsters presenting lesions was confirmed by histopathology and culture on Sabouraud dextrose-agar. Infected hamsters developed signs of disease within two to nine months; all had disseminated visceral lesions and most also had skin lesions at the sites of inoculation. None of the hamsters inoculated with skin macerates of the original hosts developed histoplasmosis, and histopathological examination of the viscera of the wild hosts failed to reveal *H. capsulatum*. Prevalence of infection was considerably higher in females than in males both for the opossum *Didelphis marsupialis* and for total wild animals (479) examined. It is proposed that canopy-dwelling mammals may acquire the infection from conidia borne on convective currents in hollow trees with openings at ground-level.

KEYWORDS: Enzootic histoplasmosis; Sex-related prevalence; Amazon Basin.

INTRODUCTION

Surveys of histoplasmin sensitivity in human populations suggest that *Histoplasma capsulatum* is widespread in the Amazon Region of Brazil^{8,10,13,17} although some of these results may have been influenced by migration. Isolation of the fungus from the native fauna is a more direct indicator of distribution, and in the Americas *H. capsulatum* has been demonstrated in the marsupials *Didelphis marsupialis*^{9,15}, *D. virginiana*⁶ and *Philander opossum*¹⁵, the rodents *Proechimys* spp^{9,15} and *Agouti paca*¹¹ the armadillos *Dasyus novemcinctus*² and *D.*

*hybridus*³ the sloth *Choloepus didactylus*⁹ as well as in several species of bats⁵ in studies in the United States^{5,6}, Panama¹⁵, Argentina³ and the Brazilian States of Pará^{2,9} and Rondônia¹¹. In this paper we present further records of *H. capsulatum* from Amazonian wildlife examined in the course of surveys to detect native hosts of leishmanial parasites. In particular, we confirm the occurrence of infection in canopy-dwelling mammals, and draw attention to an apparent female bias in the prevalence of infection.

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MATERIALS AND METHODS

Mammals were captured in live-traps, acquired from hunters, or collected manually during deforestation activities. Mammalian nomenclature follows EMMONS & FEER⁷. Tissue samples from animals sacrificed in the laboratory were prepared as previously described¹. Tissues of animals that arrived dead were triturated in 0.9% saline solution containing 5,000 units of penicillin G potassium and 5 mg of streptomycin per ml and incubated at 4° C for 2-4 hours. Liver and spleen samples triturated in isotonic saline were inoculated intradermally in the snouts and hind paws of hamsters (*Mesocricetus auratus*) as well as intraperitoneally in the same animals. Triturated skin samples were inoculated intradermally in a second group of hamsters. All hamsters were observed daily until they became moribund or died, or during 9-14 months if there was no evidence of disease. Tissues for microscopical examination were prepared as impression smears stained with Giemsa and histological sections stained with hematoxylin-eosin, periodic acid-Schiff, or Grydley. Hamster liver and spleen samples were inoculated onto Sabouraud dextrose-agar (Difco). Tissue samples from wild animals and from infected hamsters were preserved in 10% buffered formol-saline. All strains of *H. capsulatum* isolated have been cryopreserved in liquid nitrogen at INPA.

RESULTS

H. capsulatum was isolated from 28 (6%) of the 479 animals examined (Table 1). The caviomorph rodents were the group with the highest rate of infection (18%).



Fig. 1 - Lesion produced by *Histoplasma capsulatum* in the hind paw of a hamster inoculated with triturated liver and spleen of an *Agouti paca*.

TABLE I
Animals examined for *Histoplasma* infection.

Species	No. examined (No. positive)		Origin of infected animals
	males	females	
Rodentia			
<i>Agouti paca</i>	14 (1)	16 (3)	Tucuruí (PA)
<i>Dasyprocta agouti</i>	7 (0)	10 (2)	Tucuruí (PA) BR-319 km 60 (AM)
<i>Myoprocta acouchy</i>	3 (0)	6 (2)	Pitinga (AM)
<i>Coendou sp^a</i>	2 (2)	1 (2)	BR-364 km 49 (RO)
<i>Coendou prehensilis</i>	0 (0)	2 (0)	BR-364 km 49 (RO)
Marsupialia			
<i>Didelphis marsupialis</i>	191 (2)	202 (12 ^b)	Manaus (AM), Balbina (AM), BR -319 km 60 (AM), BR-364 km 42 (RO) km 354 (RO)
Xenarthra			
<i>Dasypus novemcinctus</i>	6 (0)	5 (1)	BR-364 km 49 (RO)
<i>Choloepus didactylus</i>	3 (0)	4 (1)	Tucuruí (PA)
Primates			
<i>Ateles paniscus</i>	4 (0)	2 (1)	BR-364 km 354 (RO)
TOTAL	230 (5)	249 (23 ^c)	AM, PA, RO

^a Black dwarf porcupine⁷

^b Significant difference P = 0.020 (z test).

^c P = 0.002

AM: Amazonas State, PA: Pará State, RO: Rondônia State, BR: National Highway

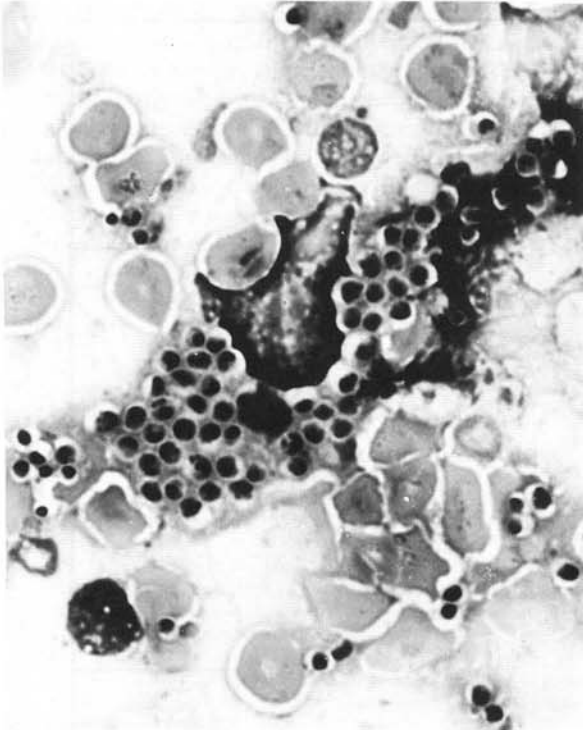


Fig. 2 - Smear of a skin lesion from a hamster showing many *Histoplasma capsulatum* cells in the cytoplasm of a macrophage. Giemsa (1,520X).

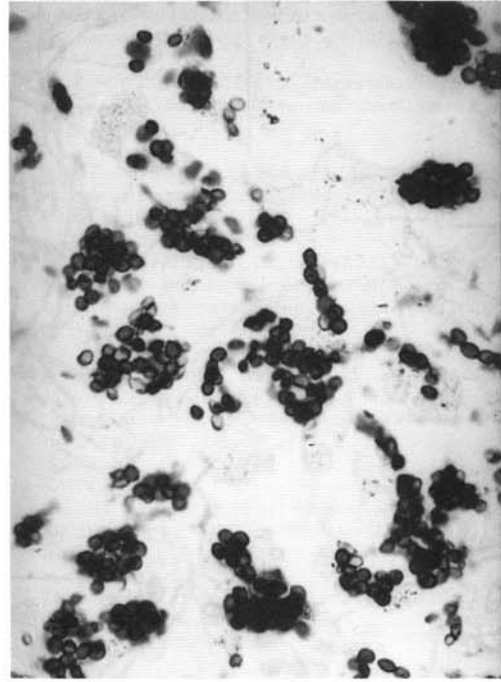


Fig. 3 - Histological section of an infected hamster liver showing *Histoplasma capsulatum* in the cytoplasm of the Kupffer cells. Grocott (1,250X).

Females presented higher infection rates than males for total animals (9% and 2%), rodents (23% and 12%), and *Didelphis marsupialis* (6% and 1%, $P = 0.020$). The infected animals did not present any macroscopic signs of histoplasmosis, nor was *H. capsulatum* detected in stained preparations of spleen, liver, lung or skin sections of these animals. None of the hamsters inoculated with skin macerates of infected animals developed histoplasmosis during the 13-14 month observation period.

Of the hamsters which became infected after inoculation of visceral macerates, most developed cutaneous lesions at the site of inoculation, after 2-8 months, although some were free of cutaneous lesions at necropsy 4-9 months after inoculation. All hamsters in which infection was detected had extensive visceral lesions. Infected hamsters (Figs. 1-3) showed large numbers of fungal cells in Kupffer cells in the liver, and in macrophages in the spleen and skin.

DISCUSSION

Foci of histoplasmosis are usually characterized by the presence of deposits of bat or bird guano, often in en-

closed spaces such as caves or attics. Our sampling sites (Fig. 4) were in widely separated areas of primary rainforest in the Brazilian Amazon, and in these areas hollow trees would appear to be the most likely foci of the fungus, as previously demonstrated in Colombia⁴ and Panama¹⁶. Hollow trees provide shelter not only for bats, but also, when there is access at ground-level, for a wide variety of mammals that inhabit the forest floor. We have observed that hollow trees that have openings at both ground and canopy levels can enclose strong convective air currents. These currents may carry fungal spores to the canopy and could account for the infections detected in sloths and spider monkeys, animals that rarely descend to the forest floor.

We have no convincing explanation for the relatively much higher rate of *Histoplasma* infection detected in female animals, which for our series of *D. marsupialis* would be expected to be observed by chance on about one occasion in fifty, if physiological or behavioural differences are not involved. In similar studies of *Paracoccidioides brasiliensis* in armadillos^{12,14}, infection rates were only slightly higher in females than in males.

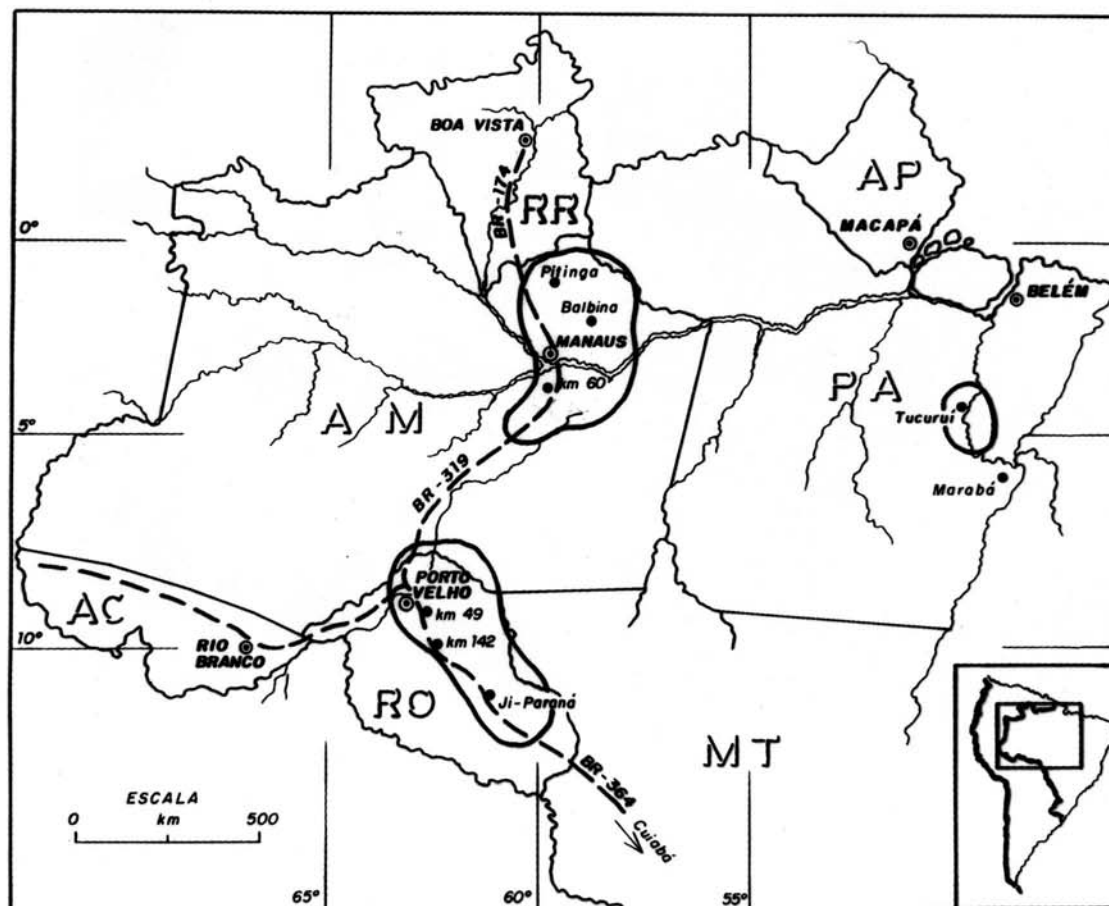


Fig. 4 - Map of northern Brazil indicating where wild animals infected with *Histoplasma capsulatum* were collected.

RESUMO

Novos registros de *Histoplasma capsulatum* em animais silvestres na Amazônia brasileira

Vinte e oito amostras de *Histoplasma capsulatum* foram obtidas de oito espécies de mamíferos silvestres nos Estados do Amazonas, Pará e Rondônia. Os isolamentos foram feitos mediante inoculação de amostras trituradas de fígado e baço em hamsters por via intradérmica e intraperitoneal. O diagnóstico micológico nos hamsters que apresentaram lesões foi confirmado por histopatologia e cultivo em meio dextrose-agar de Sabouraud. Os hamsters infectados desenvolveram sinais de doença após dois a nove meses; todos apresentaram lesões disseminadas nas vísceras e a maioria apresentou também lesões cutâneas nos locais da inoculação. Nenhum dos hamsters inoculados com material de pele dos hospedeiros originais desenvolveu histoplasmose, e

H. capsulatum não foi detectado nos exames histopatológicos dos animais silvestres. A prevalência de infecção foi consideravelmente mais alta entre fêmeas, tanto para *Didelphis marsupialis* como para o total de animais silvestres examinados (479). Propõe-se que as infecções detectadas em animais arborícolas podem ser explicadas pelo transporte de esporos do fungo em correntezas convectivas dentro de árvores ocas que tenham uma abertura ao nível do solo e outra a nível próximo das copas.

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