The Marsupial *Didelphis albiventris* is an Improbable Host of *Paracoccidioides brasiliensis* in an Endemic Area of Paracoccidioidomycosis in Minas Gerais, Brazil

ML Silva-Vergara/+, R Martinez*, MEB Malta*, LE Ramirez, FA Franco

Disciplina de Doenças Infecciosas e Parasitárias, Departamento de Clínica Médica, Faculdade de Medicina do Triângulo Mineiro, Av. Getúlio Guaritá, s/nº, 38001-970, Uberaba, MG, Brasil *Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

To determine whether Didelphis albiventris *is naturally infected with* Paracoccidioides brasiliensis, *20 specimens of this mammal were studied by both direct cultivation of their viscera (spleen, liver and lungs) and by inoculation of Swiss mice by the intraperitoneal route with a suspension of fragments of these viscera. No fungal growth or structures similar to this fungus were detected. Probably* D. albiventris *is not frequently infected with* P. brasiliensis.

Key words: *Didelphis albiventris* - *Paracoccidioides brasiliensis* - marsupial - paracoccidioidomycosis - Minas Gerais - Brazil

Despite many attempts to naturally isolate of *Paracoccidioides brasiliensis*, the ecological niche of this fungus is still a mystery for mycologists on the American Continent (Restrepo 1985). Only occasionally was this microorganism naturally recovered from soil (Albornoz 1971, Silva-Vergara et al. 1998). The isolation of *P. brasiliensis* from armadillos (*Dasypus novemcinctus*) in several regions of Brazil (Naiff et al. 1986, Bagagli et al. 1998, Silva-Vergara & Martinez 1999, Silva-Vergara et al. 2000) and, more recently, in Colombia (Corredor et al. 1999) has raised the interest in the study of this and other wild animals, especially those whose geographical distribution may overlap with that of the fungus (Restrepo-Moreno 1994).

New World marsupials belong to the family Didelphidae, which consists of 65 species included in 12 genera geographically distributed throughout the Continent (Husake 1977). *Didelphis marsupialis*, one of the most common species, has been found to be naturally infected with *Histoplasma capsulatum* on several occasions (Taylor & Shaclette 1962). Other marsupial species have also been reported to harbor this microorganism (Emmons et al. 1955, Taylor & Shaclette 1962). The objective of the present study was to evaluate *D. albiventris* as a possible host of *P. brasiliensis* in an area where this fungus was recently isolated from soil and recovered from the viscera of *D. novemcinctus* armadillos.

MATERIALS AND METHODS

With the authorization of the Brazilian Institute of the Environment (Ibama), 20 opossums (*D. albiventris*) were captured in the Triângulo Mineiro region, State of Minas Gerais, Brazil. Each animal was deeply anesthetized with 2 ml ketamine by the intramuscular route and the lungs, liver and spleen were removed under aseptic conditions, placed in distilled water containing 200 U ml^{-1} penicillin and 48 µg ml^{-1}gentamicin and stored in a refrigerator at 4ºC until the time for processing, 24-36 h later. The viscera were then manually cut into small fragments with scissors and a forceps. Parts of these fragments were cultured on Mycobiotic agar (Difco, Detroit, MI, USA) at room temperature and in Fava Netto medium (Fava Neto et al. 1961) containing 200 U ml^{-1} penicillin and 48 µg ml^{-1} gentamicin at 35ºC. A total of 60 lung fragments, 80 liver fragments and 80 spleen fragments from each animal were cultured and observed for a period of up to 12 weeks. Another part of the fragmented viscera was homogenized in sterile saline containing 200 U ml^{-1} penicillin and 48 µg ml^{-1} gentamicin. The material was used to inoculate Swiss mice by the intraperitoneal route: 7 mice were inoculated with a liver homogenate, 6 with a lung homogenate, and 7 with a spleen homogenate (20 mice for each *D. albiventris* specimen). The mice were kept in the animal house and sacrificed by exsanguination after ethyl ether anesthesia 8-12 weeks after inoculation. Liver and spleen were removed and fragmented manually. A mean num-

+Corresponding author. Fax: +55-34-3318.5279. E-mail: dip_fmtm@mednet.com.br
Received 27 November 2000
Accepted 3 May 2001

ber of 780 small tissue pieces were cultured on Mycobiotic agar at room temperature and the plates were observed for three months. Finally, another part of the viscera of 8 *D. albiventris* specimens was prepared for standard histology. Histological sections were stained with hematoxylin and eosin and with methenamine-silver (Grocott).

RESULTS

After 12 weeks of observation, no growth of colonies suggestive of *P. brasiliensis* was observed in the cultures of lung, liver and spleen fragments from 20 *D. albiventris* specimens. The inoculation of homogenates of these viscera into mice also did not permit the isolation of *P. brasiliensis*. Histopathological study of the viscera of *D. albiventris* did not show any granulomatous inflammatory reaction or fungal structures similar to *P. brasiliensis.*

DISCUSSION

The search for *P. brasiliensis* in *D. albiventris* tissues was performed by the same procedure that led to the isolation of the fungus from armadillos. In contrast to the results obtained with these animals, *P. brasiliensis* was not detected in 20 specimens of *D. albiventris.* This suggests that this marsupial is not frequently infected with the fungus or efficiently blocks the development and dissemination of the propagula of *P. brasiliensis* aspirated from the soil. By digging their holes in soil, armadillos are assumed to aspirate a larger amount of particles than *D. albiventris,* whose habits are different.

Despite this result, the search for other wild animals as hosts or reservoirs of *P. brasiliensis* is relevant, as it may lead to the habitat of this elusive microrganism.

ACKNOWLEDGEMENTS

To Maria Rita de Souza and Lúcia Helena Vital for technical assistance.

REFERENCES

Albornoz MM 1971. Isolation of *Paracoccidioides brasiliensis* from rural soil in Venezuela. *Sabouraudia 2*: 248-253.

Bagagli E, Sano A, Coelho KI, Alquati S, Miyagi M, Camargo ZP, Gomes G, Franco M, Montenegro MR 1998. Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus novemcinctus*) captured in endemic area of paracoccidioidomycosis. *Am J Trop Med Hyg 58*: 505-512.

Corredor GG, Castaño JH, Peralta A, Díez S, Arango M, McEwen J, Restrepo A 1999. Isolation of *Paracoccidoides brasiliensis* from the nine banded armadillo *Dasypus novemcinctus*, in an endemic area for paracoccidioidomycosis in Colombia. *Rev Iberoam Micol 16*: 216-220.

Emmons CW, Rowley DA, Olsen BJ, Mattern CFT, Bell JA, Powell E, Marcey EA 1955. Histoplasmosis occurrence of inapparent infection in dogs, cats and other animals. *Am J Trop Med Hyg 61*: 40-44.

Fava-Netto C, Brito T, Lacaz CS 1961. Experimental South American blastomycosis of the guinea pig. *Pathol Microbiol 24*: 192-206.

Husake II D 1977. *The Biology of Marsupials*, New York Academy Press, New York, 525 pp.

Naiff RD, Ferreira LCL, Barret TV, Naiff MF, Arias JR 1986. Paracoccidoidomicose enzoótica em tatus (*Dasypus novemcinctus*) no Estado do Pará. *Rev Inst Med Trop São Paulo 28*: 19-27.

Restrepo A 1985. The ecology of *Paracoccidioides brasiliensis* – A puzzle still unsolved. *J Med Vet Mycol 23*: 323-334.

Restrepo-Moreno A 1994. Ecology of *Paracoccidioides brasiliensis*. In M Franco, CS Lacaz, A Restrepo-Moreno, G Del Negro (eds), *Paracoccidioidomycosis*, CRC Press, Boca Raton, p. 121-130.

Silva-Vergara ML, Martinez R 1999. Role of the armadillo *Dasypus novemcinctus* in the epidemiologoy of paracoccidioidomycosis. *Mycopathologia 144*: 131-133.

Silva-Vergara ML, Martinez R, Camargo ZP, Malta MHB, Maffei CML, Chadu JB 2000. Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus novemcinctus*) in an area where the fungus was recently isolated from soil. *Med Mycol 38*: 185-191.

Silva-Vergara ML, Martinez R, Chadu A, Madeira M, Silva GF, Maffei CML 1998. Isolation of *Paracoccidioides brasiliensis* from the soil of a coffee plantation in Ibiá, State of Minas Gerais, Brazil. *Med Mycol 36*: 37-42.

Taylor RC, Shaclette MH 1962. Naturally acquired histoplasmosis in the mammals of the Panama Canal Zone. *Am J Trop Med Hyg 11*: 796-799.