Histoplasma capsulatum isolated from *Didelphis albiventris* (Marsupialia: Didelphidae) in the state of Minas Gerais, Brazil

Mario León Silva-Vergara[1], Roberto Martinez[2], Mª Helena Borges Malta[2], Claudia Maria Leite Maffei[2] & Luis Eduardo Ramirez[1]

[1]Faculdade de Medicina do Triângulo Mineiro, Uberaba, MG, and [2]Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Summary

We report the isolation of *Histoplasma capsulatum* from a culture of the viscera of *Didelphis albiventris*, one of the marsupial species found in Brazil. To our knowledge, this is the first report of the isolation of this fungus from this mammalian species. This finding confirms the ubiquitous presence of *H. capsulatum* in nature.

Key words *Didelphis albiventris*, *Histoplasma capsulatum*, Marsupial

Aislamiento de *Histoplasma capsulatum* en *Didelphis albiventris* (Marsupialia: Didelphidae) en el estado de Minas Gerais, Brazil

Resumen

En este trabajo se describe el aislamiento de *Histoplasma capsulatum* a partir del cultivo de vísceras de *Didelphis albiventris*, uno de los marsupiales encontrados en Brasil. Es la primera vez que se describe el aislamiento de este hongo en este mamífero y viene a confirmar la ubicuidad de *H. capsulatum* en la naturaleza.

Palabras clave *Didelphis albiventris, Histoplasma capsulatum*, Marsupial

Histoplasma capsulatum is a dimorphic fungus living in a saprophytic manner in the soil of environments enriched with the feces of different birds such as pigeons, grackles, oil birds, starlings, and chickens, among others [1-3]. Natural infection with this fungus has been reported for various chiropteran species [4,5], domestic dogs and cats [6,7], and wild and captive rodents and carnivores [8,9].

Histoplasmosis caused by *H. capsulatum* var capsulatum presents cosmopolitan distribution and is highly endemic in some regions of North America and in several countries in South America [10]. Ninety five percent of all infections are benign or asymptomatic. Natural infection in humans occurs by accidental inhalation of fungal propagula, frequently in the form of outbreaks [11,12].

Dirección para correspondencia:
Dr. Mario Léon Silva-Vergara
Medicina Tropical
Faculdade de Medicina do Triângulo Mineiro
Caixa Postal 118
Uberaba, MG, 38001-970, Brazil
Fax: +55 34 3318 5279
E-mail: dip_fmtm@mednet.com.br

Aceptado para publicación el 4 de Julio de 2001

New World marsupials have been found to be naturally infected with *H. capsulatum*, especially the species *Didelphis virginiana* (Virginia opossum), *Marmota mitis* (mouse opossum), *Filander opossum* (four-eyed opossum), and *Didelphis marsupialis* (commom opossum) [7,13].

Histoplasmosis is endemic in Brazil [14], (where isolation of *H. capsulatum* from rats [15], from soil [16], from dogs [17] and armadillos (*Dasypus novemcinctus)* [18] has been reported. In the present report we describe for the first time the isolation of *H. capsulatum* from *Didelphis albiventris*, one of the marsupial species existing in the country.

MATERIALS AND METHODS

With the authorization of the Brazilian Institute of the Environment (IBAMA), 20 opossums were captured in the Triângulo Mineiro region, State of Minas Gerais, Brazil, from December 1997 to August 1999. The animals were anesthetized with 2 ml ketamine by the intramuscular route and sacrificed. The lungs, liver and spleen were removed under aseptic conditions and stored in a refrigerator at 4 °C until the time for processing, 24-36 h later.

The viscera were then fragmented with scissors and a forceps on sterilized Petri dishes. The fragments were placed in culture tubes containing Mycobiotic agar® (Difco, USA) at room temperature and in Fava Netto medium [19] containing 200 U/ml penicillin and 48 µg/ml gentamicin, at 35 °C. Eighty lung fragments, 80 liver frag-

ments and 80 spleen fragments from each animal were cultured for a period of up to 12 weeks.

Part of the visceral fragments were homogenized in sterile saline solution containing 200 U/ml /ml penicillin and 48 µg/ml gentamicin. Twenty Swiss mice per *D. albiventris* specimen were inoculated intraperitoneally with 0.5 ml of the homogenate, seven of them with the liver homogenate, seven with the spleen homogenate and six with the lung homogenate. After 8-12 weeks, the mice were anesthetized with ethyl ether and sacrificed by exsanguination. The liver and spleen were removed aseptically, fragmented by hand and cultured on Mycobiotic agar® (Difco) at the average of 782 fragments per *D. albiventris* specimen. The cultures were kept at 25-30 °C and observed for 12 weeks.

RESULTS

Twenty days after seeding, the growth of a filamentous fungus arranged in small cottonwool-like colonies was observed around 60 liver fragments from one *D. albiventris* specimen. Microscopic examination of these colonies showed a morphology similar to that of *H. capsulatum* because of their filamentous aspect and typical micro and macroconidia. Yeast-like growth of *H. capsulatum* was obtained after fragments of mycelial colonies were transferred to Fava-Netto medium for culture at 35 °C. Culture of the viscera of the other 19 animals and of the organs of mice infected with their tissues did not permit the isolation of *H. capsulatum.*

To test the pathogenicity of *H. capsulatun* isolated from *D. albiventris*, an inoculum was prepared with a filamentous culture. After 15 days of growth in Sabouraud medium at room temperature, colonies from six culture tubes were fragmented and homogenized with glass beads in 25 ml of 0.9% saline, and 4.6 x 10^6 CFU/ml were obtained in the quantitative culture of this suspension. Twelve Swiss mice weighing on average 35 g were inoculated with 0.5 ml of this material by the intraperitoneal route and sacrificed between two and 12 weeks later. *H. capsulatum* was recovered in cultures of liver, spleen and lungs from the animals starting in the second week after inoculation.

To assess the antigenic reactivity of this isolate, the material was cultured in liquid McVeigh-Morton medium [20] at room temperature for 120 days. The colonies were killed with 0.001% sodium azide (Sigma, USA) and the exoantigen was obtained by filtration and centrifugation. The exoantigen was tested by agarose gel immunodiffusion with serum from patients with fungal infections: eight sera from patients with paracoccidioidomycosis, six from patients with histoplasmosis, four from patients with aspergillosis, and also with serum from patients with non-fungal infections. A precipitate line was observed with five of the six sera from patients with histoplasmosis and only one nonspecific reaction with the serum of a patient with paracoccidioidomycosis was detected. In another test, the *H. capsulatum* exoantigen was placed on an agarose gel immunodiffusion plate with sera from patients with histoplasmosis and reactivity was observed in nine of the 12 sera tested (Figure 1).

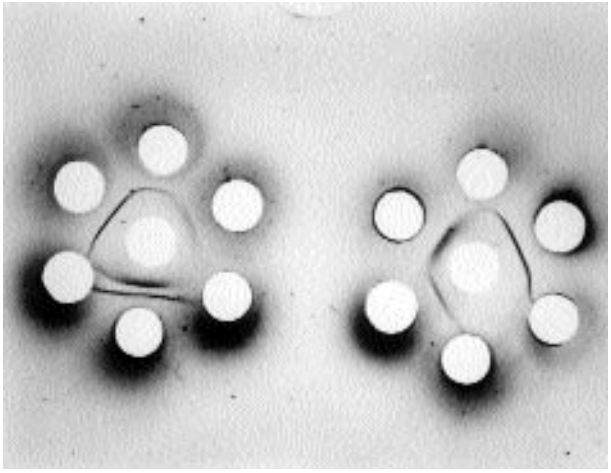

Figure 1. Agarose gel immunodiffusion-Exoantigen of *H. capsulatum* isolated of opossum (central wells) produced 1 to 2 precipitate bands with nine of 12 sera from patients with histoplasmosis (peripheral wells).

DISCUSSION

Several ecoepidemiological aspects of *H. capsulatum* have been elucidated along the last 50 years [12]. The wide geographic distribution of this fungus and its easy propagation in nature favor the infection of humans and of domestic and wild mammals [11,10].

We report here for the first time the case of a *D. albiventris* animal infected with *H. capsulatum*. New World marsupials are represented by 65 species assigned to 12 genera, all of them belonging to the family Didelphidae [21]. These mammals show a wide geographic distribution on the continent, which often overlaps with that of this fungus, thus favoring the infection of these animals, as reported for other species of this family [7,13].

The isolation of a filamentous fungus with characteristic macroconidia and showing thermical dimorphism, together with the pathogenicity demonstrated by experimental infection of Swiss mice and the positive antigenic reactivity tests with sera from patients with histoplasmosis-disease, permit us to state that the fungus isolated from *D. albiventris* is *H. capsulatum*. This finding adds another mammalian species to those naturally infected by this fungus, thus confirming the marked and already well known ubiquity of the latter. Nevertheless, it is necessary to repeat this isolation in order to find this microrganism in more than one animal.

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

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