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Paracoccidioides cerebriformis MOORE, 1935. MYCOLOGIC AND IMMUNOCHEMICAL STUDY(1)

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SUMMARY

The present study concern on mycologic and immunochemical data obtained from two samples of a fungus considered as belonging to the species *Paracoccidioides cerebriformis* described by Moore in 1935, and maintained since then on Sabouraud's agar in the mycology collection of the Instituto de Medicina Tropical de São Paulo. After 60 years, the samples exhibited the same characteristics described by MOORE (1935). However, experimental lesions did not resulted in guinea-pigs inoculated intratesticularly. The dominant antigen in *Paracoccidioides brasiliensis*, 43 kDa glicoprotein (gp43), could not be demonstrated by SDS PAGE and Western blotting. Immunoelectrophoresis did not demonstrated the E arch of cathodic migration using a policlonal anti gp43 serum. According to these findings, it is concluded that the fungus described by MOORE (1935) as *P. cerebriformis* does not belong to the genus *Paracoccidioides*. Paracoccidioidomycosis should therefore be considered as resulting from infection by a single species, *Paracoccidioides brasiliensis* (Splendore, 1912) as asserted by ALMEIDA (1930). Further studies, through molecular biology methods, could identify the mentioned fungus.

KEYWORDS: *Paracoccidioides cerebriformis*; Mycologic and Immunochemical study; Paracoccidioidomycosis.

INTRODUCTION

In 1935, on examining cultures from the fungal collection of the Instituto Oswaldo Cruz, MOORE[10] identified a new species of fungus, *Paracoccidioides cerebriformis*. The fungus was cerebriform at room temperature and was believed to cause local infection, particularly of the mouth, although rarely becoming disseminated through the body. MOORE[10,11] did not employ a particularly sensitive inoculation assay such as the guinea-pig tests for the fungus. However, ALMEIDA[1], describing the genus *Paracoccidioides* and revalidating the species *brasiliensis*, studied earlier by SPLENDORE (1912) as the agent of paracoccidioidomycosis, showed virulence in the guinea-pig, as demonstrated by parasitary orchitis. While describing *P. cerebriformis*, MOORE[10] eluded to a third species later denominated *Paracoccidioides tenuis* MOORE[12]. This species is considered by DEL NEGRO et al.[6] as typical of *Paracoccidioides brasiliensis*

In his description of the new species of *Paracoccidioides*, MOORE [10] reported that the fungus appeared in the tissues as spherical to ovoid cells, measuring 3 to 30 μm in diameter, with multiple buds or simple gemmulation. The cultures in various media were cerebriform, dull, and light brown to cream colored. Optimum growth temperature was 30°C, reduced at 37°C. Microscopically, MOORE[10] observed irregular, septate, ramified hyphae, as well as arthroconidia and chlamydoconidia, the later spherical, elongate to pyriform, being either intercalated, terminal or lateral. Lateral, elongate to pyriform conidia measure 3 to 10 μm in the long axis. The cultures did not liquify gelatin or ferment sugars, oncoagule milk which became slightly acidified. Yeast-like variants of *P. brasiliensis* obtained at room temperature have been reported, revealing that the dimorphism of the fungus does not depend exclusively on incubation temperature; rounded cells with multiple buds appear, similar to

(1) Morris Moore was a mycologist at Barnes Hospital in St. Louis, Missouri, and at the Washington University School of Medicine in the Department of Dermatology (headed by Prof. M.F. Engman). As a Guggenheim scholar, Moore visited various Latin American countries between 1935 and 1936, working at the Instituto Oswaldo Cruz (with Olympio da Fonseca Filho), at the Medical School of the Universidade de São Paulo (with Floriano Paulo de Almeida) and in the Mycology Laboratory of the Instituto Bacteriológico de Buenos Aires (with Pablo Negroni).

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those found in material collected directly from the colonies, as registrated by VILLAR et al.[15] Fungi with these characteristics are encountered in sample #741 and #750; unfortunately, which sample MOORE[10] used to describe the species *cerebriformis* is not known.

The cerebriform colonies of *P. brasiliensis* which are wrinkled at room temperature are not seen frequently in samples of the fungus, the thermal dimorphism of which is well known and well studied. The effect of temperature on such cultures has been emphasized by ALMEIDA[2] who also studied the supposed *Paracoccidioides cerebriformis*, showing that the addition of honey (containing levulose) to Sabouraud's agar resulted in the vigorous cerebriform growth of the fungus with scare mycelial filaments. The fungus exhibited a folded and irregular nature, its components "dividing and disposed one on top of the other like a budding flower". In maltose medium the filamentous form predominated.

The purpose of this paper is to verify if #741 and #750 strains of *P.cerebriformis*, first described by MOORE (1935), really belong to the genus *Paracoccidioides*.

MATERIAL AND METHODS

Two cultures, #741 and #750, kept on Sabouraud's agar, and labelled by MOORE[10] as *Paracoccidioides cerebriformis* were studied using the follow protocol:

a) Macroscopic examination of the colonies on Sabouraud's agar at room temperature;

b) Direct microscopic examination, and examination of cultures on glass slides;

c) Inoculation of approximately 0.5 mL of a *P. cerebriformis* suspension in guinea-pig testicle;

d) Production of an exoantigen in NGTA medium (1.6% neopeptone, 1% glucose, 0.01% thiamine, 0.2% asparagine) according to GARCIA et al.[7] Growth with shaking for 10 days at 25°C. The cultures were filtered through Whatman n° 1 filter paper and the filtrate concentrated 10-fold in polyethyleneglycol of 15,000 to 20,000 MW, and dialyzed for 72 hours against deionized water. Protein concentration was 10 and 8.87 mg/mL in samples #741 and #750, respectively, estimated according to LOWRY[9] method.

e) Double Ouchterlony immunodiffusion with serum from paracoccidioidomycosis patients and hyperimmune anti-gp 43 rabbit serum;

f) SDS PAGE according to LAEMMLI'S[8] method. Gel staining with the silver nitrate method, according to ANSORGE[3];

g) Western blotting according to TOWBIN et al.[14] with sera from paracoccidioidomycosis patients, hyperimmune anti-gp43 rabbit serum and policlonal anti-*P. brasiliensis* antibodies.

h) Immunoelectroforesis tests, according to SIQUEIRA[13], utilizing sera from paracoccidioidomycosis patients, hyperimmune anti-gp43 rabbit serum and policlonal anti-*P. brasiliensis* antibodies.

RESULTS

The two strains of *P.cerebriformis*, #741 and #750, cultured on Sabouraud's agar at room temperature, exhibited colonies with very similar macroscopic and microscopic characteristics. At both room temperature and at 37°C, the elevated colonies are cream colored, cerebriform in aspect, and pleated with irregular borders, becoming darker with age (Fig. 1). Microscopic examination revealed no diagnostic features permiting distinction. The elongate to pyriform hyphae are irregular and septated with arthroconidia and clamydoconidia.

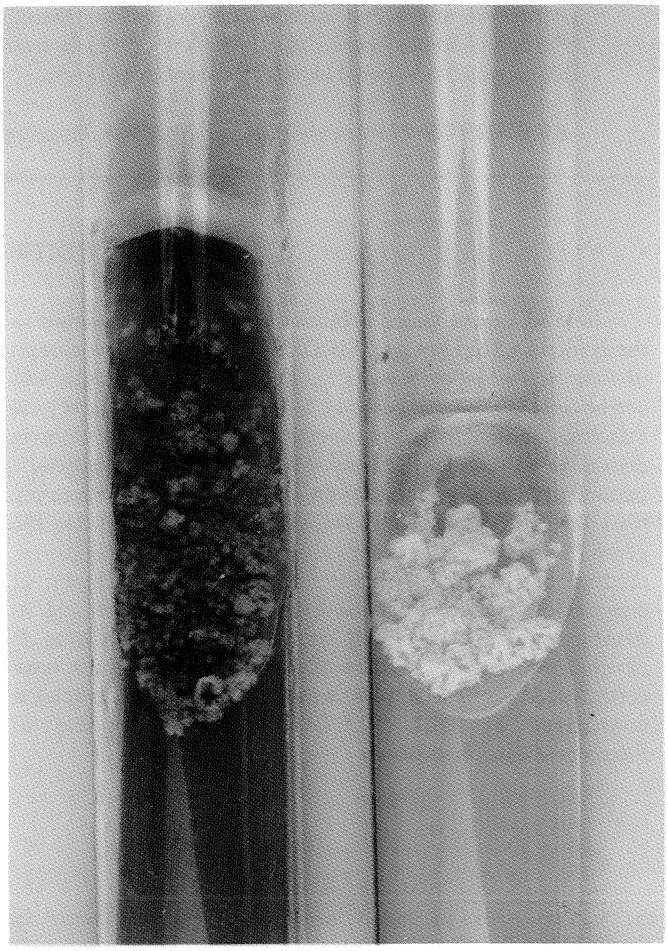

Fig 1 – *Paracoccidioides brasiliensis*. Sample #741 after 45 days of culture on Sabouraud's agar at room temperature. Sample # 750 on agar-potato medium after 20 days culture at room temperature.

Orchitis did not develop in guinea-pigs 30 days after testicular inoculation. Fungal structures characteristics of *Paracoccidioides* were absent. Sections of the testicles and epidymis showed normal histological characteristics.

Immunodiffusion was negative for anti-gp43 serum and the sera of paracoccidioidomycosis patients and policlonal anti-*P.brasiliensis* antibodies. The SDS PAGE test (Fig.2) showed a

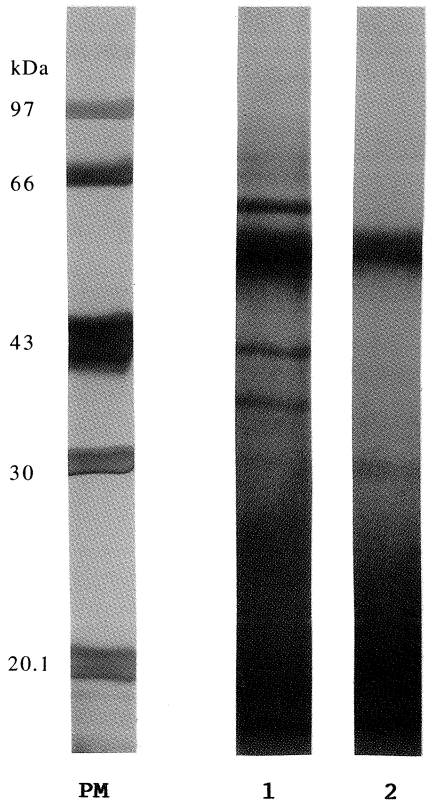

Fig. 2 – SDS-PAGE with the antigen of *P. cerebriformis*. PM, molecular weight standards; 1, sample #750; 2, Sample #741.

reduced band corresponding to gp43 in sample #750 although Western blotting was negative, this fraction not being recognized by anti-gp43 serum. The cathodic migration arch was absent with policlonal anti-*P.brasiliensis* antibodies.

DISCUSSION

The present data show that the strains or samples considered to be *Paracoccidioides cerebriformis* by MOORE[10] do not in fact belong to the genus *Paracoccidioides*. Their inclusion as one of the agents of paracoccidioidomycosis thus can be discarded, as can the notion presented by the American mycologists that the species could produce oral lesions with a limited degree of infection. The absence of pathogenicity in guinea-pigs inoculated intratesticularly may result from the lengthy period (approximately 60 years) the fungus remained in the saprophytic form, loosing its virulence over these years.

However, from the immunochemical point of view, the presence of the 43 kDa antigen, one of the dominant antigens in *P.brasiliensis*, could not be demonstrated.

These data also confirm recent findings by ASSIS et al.[5] and the paper published by ARTAGAVEYTIA-ALLENDE & MONTEMAYOR[4].

Further studies, through molecular biology methods, could identify the mentioned fungus.

RESUMO

Paracoccidioides cerebriformis Moore, 1935. Estudo micológico e imunoquímico

O presente estudo trata dos resultados obtidos, do ponto de vista micológico e imunoquímico, de duas amostras de *Paracoccidioides* consideradas como pertencentes à espécie *cerebriformis*, criada por MOORE in 1935 e mantidas desde aquela época, através de repiques em ágar-Sabouraud, na Micoteca do Instituto de Medicina Tropical de São Paulo. Após cerca de 60 anos, tais amostras conservavam as mesmas características descritas por MOORE (1935). Não foram registradas lesões experimentais em cobaios inoculados por via intratesticular, não se demonstrando, também, pelas técnicas de SDS PAGE e Western blotting, o antígeno dominante do *Paracoccidioides brasiliensis*, representado pela glicoproteína de 43 kDa (gp43). A imunoeletroforese também não revelou o arco E de migração catódica, utilizando-se um soro policlonal anti-gp43. Em vista desses resultados, concluímos que *Paracoccidioides cerebriformis* não pertence àquele gênero, devendo-se considerar a paracoccidioidomicose como infecção fúngica causada por uma única espécie de *Paracoccidioides* - *Paracoccidioides brasiliensis* (SPLENDORE, 1912) ALMEIDA, 1930.

No futuro, através de métodos de biologia molecular, talvez possamos identificar este fungo.

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