

"PARACOCCIDIOIDOMYCOSIS-INFECTION" SURVEY IN BRAZILIAN CAPTIVE PRIMATES (*Cebus apella*)

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SUMMARY: The aim of this study was to verify the possible occurrence of paracoccidiodomycosis-infection in animals that belong to the Order Primates, once they are phylogenetically closest to the only naturally susceptible host of the disease, the human being. This research was essayed using paracoccidiodin delayed hypersensitivity test in 33 *Cebus apella*, "weeping-capuchin". Paracoccidiodin positive skin reactions were detected at a rate of 33.33%. The humoral immunological response was detected by complement fixation test in 10% of the performed sera. Histological examinations of the intradermic test biopsies showed the presence of focal or nodular dermatitis deep in the dermis, prevailing the polymorphonuclear neutrophils both at 24 and 48 hours after inoculation, with oedema, congestion, and mild vasculitis. The results obtained in this study indicated that Cebidae are susceptible to natural "paracoccidiodomycosis - infection" and are suitable to be used in epidemiological surveys.

UNITERMS: Mycoses, paracoccidiodomycosis; Paracoccidiodomycosis; South-America blastomycosis Primates

INTRODUCTION

Paracoccidiodomycosis is a systemic infectious disease geographically restricted to Latin America, caused by a dimorphic fungus, *Paracoccidioides brasiliensis*²². The disease is characterized by the development of granulomatous lesions in the lungs, skin, mucous membranes and other tissues. It is one of the most important deep mycosis by its high prevalence, and by the death of the patient¹⁶.

It is primarily a disease of human being. Indeed it was not described in other species except the isolation of this fungus from tissues of four trapped armadillos (*Dasypus novemcinctus*)²⁰ and the histological finding of suggestive structure of *P. brasiliensis* in granulomatous lesions of colon and liver of a female monkey (*Saimiri sciureus*)¹⁵.

On the other hand, many animal species are susceptible to experimental infection with *P. brasiliensis*, e.g. the common laboratory animals like mice (*Mus musculus*)^{4,18}, guinea pig (*Cavia porcellus*)¹, hamster (*Mesocricetus auratus*)^{4,14}, and rabbits (*Oryctolagus cuniculus*)²¹. Among the wild animals, experimental infection with this fungus was performed in marmosets (*Callithrix sp*)²¹, "Casiraguas" (*Proechimys guayanensis*)² and bats (*Arabeus lituratus*)¹². Even the domestic animals are susceptible to experimental paracoccidiodomycosis, as the cat⁴, dog^{4,19} and cattle⁶.

The study of paracoccidiodomycosis infection in animals through intradermic test was performed in equines³, bovines¹³ and in these two species and also in the ovines⁵.

Considering that paracoccidiodomycosis is the most prevalent human deep mycosis in Latin America²² and the paradoxical lack of knowledge of its occurrence among the animals, the aim of this research was to study the occasional occurrence of paracoccidiodomycosis-infection in specimens belonging to the Order Primates. Since these animals phylogenetically are the closest to the only natural susceptible host of the disease, the human being, it seems that this objective is fully justified.

MATERIAL AND METHOD

Animals: a total of 33 adult specimens of weeping-capuchin "macaco prego" (*Cebus apella*), Cebidae, Order

Primates, of both sexes, kept in captivity* were used in this study.

Paracoccidioides brasiliensis antigens:

- 1- Polysaccharide (PP). Obtained from seven strains (18, SM, AS, 192, 265, SN, Adelino) of *P. brasiliensis*, from the collection of the Department of Microbiology and Immunology of the "Instituto de Ciências Biomédicas da Universidade de São Paulo", prepared as described by FAVA NETO et al.¹⁰ (1969). This antigen was used for intradermic tests, precipitin and complement-fixation reactions.
- 2- Yeast cell suspension (PS). An equal volume of yeast cells from each of the *P. brasiliensis* strains, after the extraction of the polysaccharidic antigen, was used to obtain a concentrated suspension, as described by COSTA⁵, 1975. For the intradermic tests a dilution standardized by tube 5 of the Mac Farland scale was used.

Tuberculin: PPD (Purified Protein Derivative) from SERUM Institut of Copenhagen Lot NT23, standardized dilution 2 UI/0.1 ml.

Intradermic tests: The intradermic tests were performed in the skin of the abdominal region. Trichotomy was made over a 4 x 5 cm area in such way that the three injections could be made 5 cm apart from each other. The antigens were injected each time in an 0.1 ml volume. The diameters of these reactions were measured after 24 and 48 hours. Five milimeters or greater diameters were considered positive reactions.

Serological tests: These were performed on 20 sera among the 33 animals studied.

- 1- **Complement fixation:** The technique of Wadsworth Maltaner & Maltaner (WADSWORTH²⁴, 1947) was employed according to the standardization made for paracoccidioidomycosis system by FAVA NETO⁸ (1977).
- 2- **Precipitin reaction:** Carried out as standardized by FAVA NETO⁹ (1961).

Histopathological study: Biopsies of positive skin tests were obtained with a four milimeter skin punch, under local anaesthesia, 24 and 48 hours after antigen injections. The materials were fixed in Bouin liquid,

embedded in paraffin and the 5 micrometers thick sections were stained with Hematoxilin and Eosin.

RESULTS

The paracoccidioidin test was positive in eleven cases of the thirty three weeping-capuchin (*Cebus apella*) tested (33.33%). The tuberculin test was positive in 6.06% of these animals. Positive reactions for both paracoccidioidin and tuberculin were not observed in the same animal.

The serological tests (complement-fixation and precipitin tests) were carried out with the sera of 20 specimens; from these, only 10% were positive, the complement-fixation test revealed low levels of antibodies in these serum, and the precipitation tests in liquid medium were all negatives. Biopsies of the positive intradermic tests, performed 24 and 48 hours after antigen infections showed different patterns of inflammatory reaction. The animals injected with the yeast cell suspension showed in the deep dermis an inflammatory infiltrate of neutrophils, some eosinophils, being seen also the *P. brasiliensis* yeast cells. In the superficial dermis a mild inflammatory infiltrate, mainly by mononuclear cells, distributed around the little vessels and cutaneous adnexa, could be observed (Fig. 1 and 2). Skin biopsies of the animals that received the polysaccharidic antigen showed a diffuse inflammatory infiltrate in the dermis with mononuclear and polymorphonuclear cells seen around the little blood vessels and cutaneous adnexa, along with oedema and collagen fibers dissociation.

DISCUSSION

In spite of the fact that many animal species are susceptible to *P. brasiliensis* experimental infection, as mentioned above, little attention has been given to the natural occurrence of paracoccidioidomycosis infection or disease in animals.

The principal aim of this study was to investigate the epidemiology of this mycosis, and to ascertain the suitability of the Primates of an endemic area to be used in an epidemiological survey. This idea is justified, as referred before, since Primates are the closest to the human being, which seems to be the most susceptible to *P. brasiliensis*. Naturally acquired paracoccidioidomycosis disease has been comprobated only in man²².

The rate of paracoccidioidin positivity (33.33%) presented by the 33 specimens of *Cebus apella* tested was higher than the tuberculin positivity (6.0%), and no cross reaction was observed, i.e., the animals which

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presented paracoccidioidin positivity were tuberculin negative.

The histopathological picture findings were compatible with a delayed type hypersensitivity reaction, described for various antigens inoculated in the skin of men²³.

Only two among the twenty tested sera (10%) showed positive complement fixation test, revealing the occurrence of low levels of antibodies in the sera of paracoccidioidin-positive animals. The same picture was observed by COSTA et al.⁶ (1978) in a survey carried out in bovines, equines and ovines.

The results obtained in this study indicated that Cebidae are susceptible to natural "paracoccidioidomycosis-infection" and are suitable to be used in epidemiological surveys.

As a matter of fact, the concept of "infection without disease" in deep mycosis was well established since 1938 by DICKSON; GILFORD⁷ (1938), and for paracoccidioidomycosis since 1959 by LACAZ et al.¹⁷ (1959). As in any other infection, deep mycosis infection is almost always revealed only through immunological studies, mainly by delayed hypersensitivity test.

Recently a new classification for the forms of occurrence of this mycosis was proposed by an international committee of experts,¹¹ which defined the polar forms as "anergic or malignant, characterized by disseminated disease, impaired cellular immune response, high levels of specific antibodies, loose granulomatous inflammation with necrosis and great numbers of fungi in the lesions", and the "hyperergic or benign form, which is localized, presents effective cellular immune response, low levels of antibodies, compact epithelioid granuloma, and absence of/or few fungi in the lesions".

The results presented by the specimens of *Cebus apella* studied were in accordance with the form classified as hyperergic or benign or, as mentioned above, they presented the paracoccidioidomycosis infection.

Although well known since the early twentieth century, paracoccidioidomycosis is the main endemic systemic mycosis in Latin America, and in some aspects they are still poorly understood, like for instance, the ecological "niche" in nature of the saprophytic phase²² and little is known about the host-parasite interactions in paracoccidioidomycosis¹¹. The participation of the animals in the epidemiological chain of this mycosis is still a matter to be elucidated.

COSTA, E.O.; DINIZ, L.S.M.; FAVA NETO, C.; ARRUDA, C.; DAGLI, M.L.Z. Pesquisa da ocorrência da

"Paracoccidioidomycose-infecção" em Primatas brasileiros, mantidos em cativeiro. *Braz. J. vet. Res. anim. Sci.*, São Paulo, v.29, n.1, p.39-44, 1992.

RESUMO: O objetivo deste trabalho foi estudar alguns aspectos da epidemiologia desta micose, pesquisando a ocorrência de paracoccidioidomycose-infecção em animais da Ordem Primata, uma vez que são filogeneticamente os mais próximos ao homem, único hospedeiro naturalmente susceptível a esta micose sistêmica, no atual estágio de conhecimento. Foram realizados testes de hipersensibilidade do tipo tardio com paracoccidioidina em 33 exemplares de *Cebus apella* (macaco prego), obtendo-se 33,33% de positividade. Foram também executadas biópsias de reações intradérmicas para exame histológico, testes sorológicos, de fixação de complemento e precipitação em meio líquido no soro destes animais. Os resultados obtidos permitiram verificar a ocorrência de paracoccidioidomycose-infecção em primatas não humanos, sugerindo a possível participação destes animais na epidemiologia da paracoccidioidomycose.

UNTERMOS: Micoses, paracoccidioidomycose; Paracoccidioidomycose; Blastomicose Sul-americana; Primatas

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FIGURE 1 — Intradermic test at 48 hours with paracoccidioidin yeast cell suspension. Focal inflammatory reaction in the deep dermis and mild vasculitis in superficial dermis. (H.E. 165 x).



FIGURE 2 – Higher magnification of Fig. 1. *P. brasiliensis* yeast cells of the antigen and polymorphonuclear inflammatory cells. (H.E. 660 x).