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In Vivo *Paracoccidioides* sp. Biofilm on Vascular Prosthesis

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Abstract Paracoccidioidomycosis is an endemic mycosis caused by *Paracoccidioides* species limited to Latin America arising with the chronic form in 90% of cases. The capacity of microorganisms to form biofilms is considered of great importance medical since can contribute to the persistence and to the chronic state of the diseases. The ability of *Paracoccidioides* to form biofilm has been demonstrated in vitro. In our study, for the first time we have observed this capability in vivo on a vascular prosthesis using scanning electron microscope showing a dense network of *Paracoccidioides* yeasts covered by an extracellular matrix.

Keywords Biofilm · Endemic mycosis · Paracoccidioidomycosis · Dimorphic fungi

Paracoccidioidomycosis (PCM) is an endemic mycosis limited to Latin America, caused by *Paracoccidioides brasiliensis* with three different phylogenetic groups (PS1, S2, S3) and *Paracoccidioides lutzii* [1].

Argentina has two endemic areas of PCM, one in the northwest and the largest in the northeast of the country where Chaco province has the highest incidence of PCM arising with the chronic form (adult type) in 90% of cases [2].

A 63-year-old man from Chaco (Argentina) who required an aortobifemoral vascular prosthesis as a result of Leriche syndrome suffered an intestinal infarct and acute ischemia in the right lower limb by septic emboli two years later. Consequently, an axillofemoral bypass and extraction to the infected prosthesis were performed. The extracted prosthesis was sent to the Servicio de Microbiología of Perrando Hospital for microbiological study. Numerous multibudding yeast cells of *Paracoccidioides* sp. were observed in direct examination (Fig. 1). The diagnosis was disseminated PCM [3].

There is a considerable interest in biofilm formation because it is crucial for the development, spreading and the persistence of infection diseases [4, 5]. Since the knowledge about the *Paracoccidioides* biofilm formation is scarce, the aim was to investigate whether *Paracoccidioides* was able to form biofilm in vivo on the vascular prosthesis using scanning electron microscope.

A portion of Dacron prosthesis was fixed in FAA (formalin–acetic acid–alcohol) solution during 24 h. Following fixation, the specimen was dehydrated

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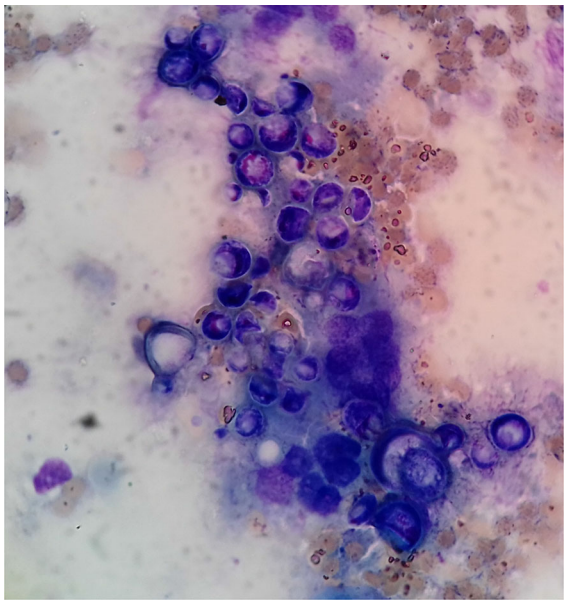

Fig. 1 Numerous multibudding *Paracoccidioides* yeasts from a portion of aortobifemoral vascular prosthesis (Dacron). Giemsa stain (×1000)

using a series of gradient ethanol–acetone solutions and critically point dried in CO_2. Then, gold coating was performed. Sample was examined under a Joel 5800 LV (Tokyo, Japan) scanning electron microscope at Servicio de Microscopía Electrónica (Universidad Nacional del Nordeste, Argentina).

It has been recognized that adhesion of microbes to surfaces is an important factor for colonization and plays a decisive role in the pathogenesis of microbial infection [5–7]. A wide variety of fungi have demonstrated their ability to colonize surfaces and form biofilm. Most studies on fungal biofilms were focused on *Candida albicans* and other fungi [6, 8].

Sardi et al. [4] have demonstrated in vitro the ability of *Paracoccidioides* to form biofilm. In our study, we have observed in vivo this capability. Figure 2 shows a dense network of *Paracoccidioides* yeasts covered by an extracellular matrix forming biofilm adhered to the aortobifemoral vascular prosthesis portion.

The capacity of the fungus to form biofilms was highlighted as being of great medical importance. This biofilm may contribute to the persistence of infection and to the chronic state of the disease. Paracoccidioidomycosis is endemic in all Latin-Americans countries with 90% of cases of the chronic form. Furthermore, the mature biofilm with extracellular

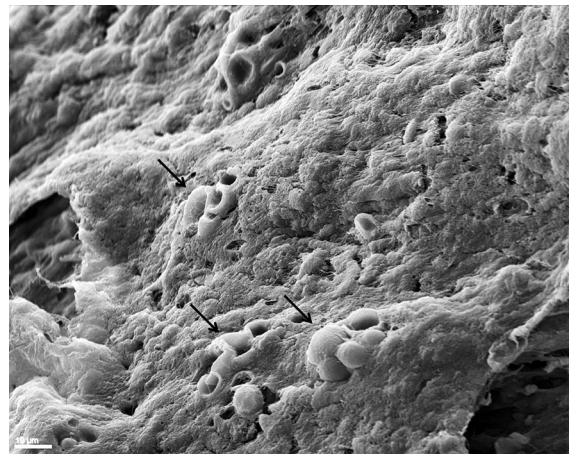

Fig. 2 Multibudding *Paracoccidioides* yeasts and extracellular matrix (*arrows*) on aortobifemoral vascular prosthesis (Dacron) under a Joel 5800 LV (Tokyo, Japan) scanning electron microscope (SEM) (×750)

matrix observed, where the pathogen is confined, can become a reservoir of cells that can hinder the action of antifungal drugs and avoid the host defenses making it difficult eradicate the infection [4, 5].

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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