

TITLE: *Paracoccidioides brasiliensis* DNA ON SOIL SAMPLE FROM SOUTHERN BRAZIL

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ABSTRACT:

In the last years paracoccidioidomycosis disease and infection has been described in Southern Brazil, corroborating with the data that *Paracoccidioides* spp. have had their endemic areas expanding. Thus, this study aimed to report the DNA detection of *Paracoccidioides brasiliensis* in a soil sample from the southern Brazil. Two soil samples from Rio Grande, Rio Grande do Sul, Brazil, stored at the Laboratory of Mycology of the Faculty of Medicine, Federal University of Rio Grande, were analyzed. The DNA extraction Norgen Biotek® Kit (Canada) was used according to the manufacturer's instructions, and molecular detection was performed using the Nested-PCR technique with ITS4 and ITS5 as external primers, PBITS-E and PBITS-T as internal primers. One of the samples was experimentally contaminated with a inoculum of the standard strain Pb18 as positive extraction control. Also, as positive and negative PCR controls, we used the DNA of the Pb18, extracted in house directly from the culture, and the DNA from a strain of *Sporothrix brasiliensis*, respectively. DNA amplification product of about 424 bp compatible with *Paracoccidioides* spp. was found in both soil samples and in the positive control PCR reactions. To the identity confirmation of the fungus, the DNA product was purified and sequenced using Applied Biosystems 3500 Genetic Analyzers, showing 100% of identity with the internal transcribed spacer of *P. brasiliensis* Pb18. Since the isolation of the fungus directly from the soil is very difficult, molecular techniques become essential for the knowledge of the *Paracoccidioides* spp. ecoepidemiology. Thus, this study represents an important step, showing for the first time the detection of *Paracoccidioides* spp. DNA in a soil from south of Rio Grande do Sul, Brazil, and instigates further studies to a better understand of the ecoepidemiology of PCM agents in this area characterized by the pampa biome.

Keywords: soil, *Paracoccidioides* spp., Nested-PCR, Rio Grande do Sul

Development Agency: CAPES, Universidade Federal de Pelotas and Universidade Federal de Rio Grande