Molecular epidemiology of *Paracoccidioides brasiliensis*. Preliminary report of the Argentinian –Paraguayan Multicentric Study of *Paracoccidioides* and Paracoccidioidomycosis

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Paracoccidioidomycosis (PCM) is the most important systemic fungal infection in Latin America. Primary pathogenic dimorphic fungi *Paracoccidioides brasiliensis* and *P. lutzii* are the etiological agents. Genetic diversity between phylogenetic species was reported. P. brasiliensis contains a complex of at least four different cryptic species (S1, PS2, PS3 and PS4). A great number of phenotypes may overlap in these related groups, discouraging the use of morphology alone for species recognition.

Since 2014 an Argentinian-Paraguayan Multicentric study of *Paracoccidioides* and Paracoccidioidomicosis we have been conducting.

In the present study, using PCR-RFLP of the alpha-tubulin (TUB1) gene, 35 strains were processed in order to identifying cryptic *Paracoccidioides* spp.

DNA was extracted from 14-day colonies using boiling–thermic shock combination described by Giusiano et al.

Alpha-tubulin gene fragments were amplified using primers α-Tub F (5-CTG GGA GGT ATG ATA ACACTG C-3) and α-Tub R (5-CGT CGG GCT ATT CAGATT TAA G-3) described by Roberto et al.

A portion of the TUB1 gene was amplified and double digested in vitro using *Bcl* I and *Msp* I endonucleases, which generates four different electrophoretic patterns corresponding to the four main genetic groups: S1, PS2, and PS3 of *P. brasiliensis* and *P. lutzii*.

As references, 11 strains previously sequenced and classified by WGS were used. *P. brasiliensis* S1 (n=33; 94.3 %) was the major group recognized followed by PS2 (n = 2; 5.7 %).

Except for one strain (PbP99) misidentified by TUB1-RFLP, a good agreement with WGS was observed.

TUB1-RFLP is a fast and inexpensive tool for identifying *Paracoccidioides* spp., which can be used as a screening method to apply in molecular epidemiological studies of paracoccidioidomycosis.

Keywords: *Paracoccidioides*, PCR-RFLP, Molecular identification