

NEW RECORDS AND RESEARCH OF *Paracoccidioides brasiliensis* AND *P. lutzii* IN ARMADILLOS AND SOIL SAMPLES, FROM SÃO PAULO AND MATO GROSSO.

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Summary:

Paracoccidioides brasiliensis (Pb) and *P. lutzii* (Pl) (Onygenales, Ascomycota) are responsible for paracoccidioidomycosis (PCM), an important systemic mycosis in Latin America. They have saprobic phase in soil and parasitic phase in tissues of vertebrate hosts. Ecological studies involving Pb and its association with the nine-banded armadillo, *Dasypus novemcinctus* are promising to map areas of risk to PCM and identify which of the phylogenetic species of Pb (S1, PS2) are present in the environment. The association between armadillos and Pl has not yet been demonstrated. Therefore, we proposed to isolate and molecularly identify the *Paracoccidioides* species from armadillos, and molecularly detecting the fungus in the animal tissues and in soil samples from Botucatu/SP region and Alta Floresta/MT. With the approval of CEUA and IBAMA, two armadillos from Botucatu / SP were evaluated for spleen culture (S), liver (L) and mesenteric lymphnodes (ML) in Mycosel Agar. We also evaluated 06 soil samples (03) from SP and 03 from MT) collected inside and outside armadillo burrows, closed to forest and in grassland areas. The DNA of soil samples and animal tissues were obtained by using commercial kits and subjected to nested PCR reactions with specific primers for both species; and the amplicons were also sequenced and compared on BLASTn. In a total of 581 fragments of S, 1.936 of L and 206 of ML, Pb was isolated in two armadillos, in 11 ML fragments. The molecular characterization (sequencing of ITS-5.8S region) showed 99-100% of similarity for Pb, in the armadillo samples from SP, as well as in soil samples from SP and MT. Pb was detected in soil samples collected in the forest from both regions (SP and MT), while it was negative when collected in the pasture areas. The data reinforces the importance of armadillos, especially the mesenteric lymphnodes, for fungal recovery and ecological mapping of the pathogen. The occurrence of the *P. lutzii* in armadillo has not been confirmed until now.

Key words: molecular detection, Paracoccidioidomycosis, Nested-PCR.

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