

TITLE: *Cryptococcus gattii* AND *C. neoformans*: PHENOTYPIC DIVERSITY AND VIRULENCE IN MURINE MODEL

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ABSTRACT:

Cryptococcus gattii and *C. neoformans* are the main species of the genus *Cryptococcus* that have medical and veterinary relevance. These microorganisms are ubiquitous and, in the environmental and host situation, face many stresses, such as temperature, pH, osmolarity, low nutrient availability, among others. This study evaluated the variation in virulence and pathogenicity attributes of five strains of *C. gattii* and five of *C. neoformans*, in vitro and in vivo. It was demonstrated that the morphology and development of *Cryptococcus* are extremely variable according to the culture conditions (medium and temperature) and seldom, changes observed in vitro, occur in vivo. It was found that *C. gattii* and *C. neoformans* culture in nutritionally poor media, such as the Minimum Liquid Medium (MLM) and Minimum Liquid Medium supplemented with fetal bovine serum (MML + SFB10%), causes significant reduction ($p < 0.05$) of the cell diameter, increase ($p < 0.05$) the surface volume ratio (S / V) and capsular thickness if compared to the rich medium Sabouraud liquid dextrose (SLD). This morphological variation turns the cell into an able system that optimizes its metabolism and increase its reproductive fitness. For *C. gattii*, the WM179 strain exhibited the highest capsular thickness in the MLM, as well as for the WM628 and WM626 strains of *C. neoformans*. In the next step the virulence in murine model of infection was investigated. However, the increase of the capsule in the in vitro system was not determinant for virulence, once in the lethality analyzes, the WM179 strain exhibited low virulence and had reduced fungal load in the lung and was undetectable in the brain after 10 days of infection. Immediately after infection, there is a significant reduction of the cell body, both for *C. gattii* and *C. neoformans*, we believe that this factor can favor the passage through the blood-brain barrier and access to the CNS at the beginning of the infection. Then, a relation was observed between increase of the polysaccharide capsule in the lung and bronchoalveolar lavage during the infection with the virulence of *C. neoformans* strains, but it was not found in *C. gattii* strains. It is concluded that the virulence of *C. neoformans* and *C. gattii* is associated to the phenotypic diversity of this microorganism, mainly for *C. neoformans*, in which morphological variations in vivo were able to predict the virulence of each lineage.

Keywords: Criptococose, phenotypic diversity, virulence

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