Pathogenicity of environmental strains of Cryptococcus neoformans var neoformans in murine model

Dear Editor,

During an investigation on the epidemiology of cryptococcosis in Belgium, many isolates of Cryptococcus neoformans var. neoformans were recovered from various environmental substrates. The present study was undertaken to verify the pathogenic potential of all the 70 strains of C. neoformans in a mouse model by intracerebral route. Conventionally bred white mice (NMRI) of both sexes (3-5 weeks old) were used. All the 70 saprobic isolates of C. neoformans var. neoformans were cultured on Sabouraud dextrose agar at 25 °C, and 48 h old growth was suspended in sterile saline solution to contain approximately one million yeast cells. Each strain was inoculated in two mice; and each animal received $10^6$ organisms in 0.05 ml suspension intracerebrally. Most of the mice died within 11-44 days due to heavy multiplication of the fungus in the brain, showing the symptoms of dullness, depression, anorexia, raised skull (dome shaped), lowering of head, weakness, circling movement and ataxic gait. However, 31 mice failed to exhibit any clinical signs of the disease even after 45 days. These mice were sacrificed after 45 days. At necropsy, the brain of most animals showed slimy, mucoid and gelatinous masses of different sizes. Squash preparations of the infected brains revealed aggregates of C. neoformans cells with thick, enlarged and wide capsules. The tissue imprints also showed many capsulated organisms of C. neoformans in PAS stain. Numerous fungal cells of C. neoformans embedded in the brain parenchyma were also demonstrated by Mayer’s mucicarmine technique. The organism was reisolated from the brain tissues of all the 140 mice on Pal’s sunflower seed medium (pulverized sunflower seed 45 g, agar 20 g, chloramphenicol 100 mg, distilled water 1000 ml, pH 5.6 to 5.8 at 25 °C). The results of this study indicated that isolates of C. neoformans vary in their pathogenicity to mice. As cryptococcosis is a cosmopolitan, highly infectious and enigmatic mycosis, the susceptible hosts, particularly immunocomprised patients, must take preventive measures for protection against exposure to C. neoformans, which grows in a wide variety of environmental materials.

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