Title: STANDARDIZATION OF REAL-TIME PCR TECHNIQUE FOR DETECTION OF *Histoplasma capsulatum* IN BATS FECAL SAMPLES IN THE MUNICIPALITY OF SÃO PAULO

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Abstract:

The concentration of the Brazilian population in urban areas has transformed these areas into a high complex environment. The emerging and reemerging of certain diseases are associated with the environmental degradation, which allows adaptation of pathogenic reservoirs, vectors and microorganisms. This is the case of some wild populations, such as bats, which tend to reside and proliferate in urban environment. This work aims to identify *Histoplasma capsulatum* in bats fecal samples collected in residences in the urban area of São Paulo, using the technique of real-time PCR. A total of 13 samples were used in this work: 2 samples of *H. capsulatum* culture from the collection of fungi of the Mycology sector of Labzoo - CCZ (MIC190/03 and MIC1408/14-positive control), 10 samples of bats feces, and 1 feces sample added with *H. capsulatum* (MIC 1408/14). The fungal DNA extraction was carried out using the PowerSoil Isolation Kit (Mo Bio) for culture samples, and the PowerFecal Isolation Kit (Mo Bio) in case of fecal samples. The real-time PCR assay targeted the ITS1 region of the ribosomal DNA and used the specific primers HcITS-54F (5'-ACCCTTGTCTACCGGACCTGTT-3') e HcITS-204R(5'-TTTTGACTGGATTATTATCGCTCTCA-3') and probe HcITS-155 (5'- FAM-CGGTGAACGATTGGCGTCTGAGC- QSY 3'). All reactions were run for 10min at 95°C and 40 cycles as follows: 15s at 95°C and 1min at 60°C on the StepOne PCR System (Applied Biosystems). The samples were also analyzed by conventional PCR. However, the real-time PCR technique demonstrated a greater sensitivity than the conventional PCR method and correctly identified *Histoplasma capsulatum* in the culture-positive feces sample (with added *H.capsulatum*) and in the culture extracts. Of the 10 samples of feces reviewed, only one presented amplification curve, suggesting that this sample is positive. On the other hand, in conventional PCR, only the positive control was visualized in agarose gel. This protocol seems to be a promising tool to efficiently detect this pathogen in environmental samples. The detection of pathogenic microorganisms in clinical and environmental samples is essential to diagnose and prevent diseases. Therefore, by identifying environmental reservoirs of microorganisms, we can trigger actions to promote the health of the population.

Keywords: Public Health, Environment, Real-Time PCR, *Histoplasma capsulatum*
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