RESUMO SIMPLES - OUTRAS ÁREAS TEMÁTICAS

CYNICLOMYCES GUTTULATUS IN CATS: A MOLECULAR SCREENING STUDY

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Introduction: Cyniclomyces guttulatus is a yeast traditionally described as a gastrointestinal commensal in rabbits, but increasing detection in dogs and cats has raised questions about its ecological adaptability and potential significance in small animal medicine. Despite occasional reports in felines, the species remains poorly studied, especially due to the absence of molecular investigations focused on its detection in this host. Goals: This study aimed to identify C. guttulatus in fecal samples from domestic cats and to evaluate the performance of three molecular techniques targeting the RPB1 gene: external PCR, nested PCR, and nested PCR using diluted template (1:20), in order to determine their sensitivity, reproducibility, and suitability for veterinary diagnostic laboratories. Methods: A total of 240 feline fecal samples were analyzed, comprising animals with and without gastrointestinal signs. DNA was previously extracted, and all samples underwent initial screening by external PCR using RPB1-specific primers, selected for their higher taxonomic resolution compared with ribosomal markers. The first amplifications, performed with reference DNA,

produced clear bands anos detected 39 positive samples (16.25%). Positive controls were tested in different concentrations using Nested PCR. When the undiluted product of the first reaction was used, sensitivity did not increase substantially. Considering the presence of inhibitors commonly found in fecal matrices, the external PCR product was diluted 1:20 before being used as template for the nested step, and all the samples previously found negative were tested. This refinement significantly improved the clarity and intensity of the bands and resulted in 41 additional positive samples. Results: Altogether, 80 of the 240 samples (33.3%) showed detectable amplification of the expected RPB1 fragment. Throughout the entire study, negative controls remained free of amplification, confirming specificity and the absence of contamination. These findings reinforce how small methodological adjustments—such as diluting the template—can meaningfully increase sensitivity without complicating the protocol or raising costs. The high number of positive samples suggests that C. guttulatus may be more common in the feline intestinal microbiota than previously assumed. Because detection occurred in both symptomatic and asymptomatic cats, the yeast may behave as a transient or stable commensal rather than a primary pathogen, consistent with previous observations in dogs. Nevertheless, the consistency of detection across independent reactions indicates that its presence may represent true colonization rather than incidental ingestion. Conclusions: Overall, the study highlights the RPB1 gene as a reliable marker for identifying C. guttulatus in felines and supports the diluted nested PCR as the most sensitive and practical method among those tested. The optimized protocol is low-cost, reproducible, and capable of detecting low fungal loads, making it applicable to routine veterinary diagnostics. Future studies incorporating gene sequencing, phylogenetic comparison with isolates from various host species, and longitudinal monitoring of positive cats will be essential for determining the ecological role, persistence, and potential clinical relevance of this yeast in companion animals.

Palavras-chave: cyniclomyces guttulatus; feline medicine; rpb1; nested pcr; fungal detection.