

## MOLECULAR IDENTIFICATION OF *Prototheca* STRAINS ISOLATED FROM BOVINE MASTITIS CASES IN SÃO PAULO STATE, BRAZIL

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### Abstract:

Bovine mastitis caused by microalgae of the genus *Prototheca* has become a serious problem for the dairy industry worldwide. *Prototheca* intramammary infections are refractory to mastitis antimicrobial therapy and can result in outbreaks, chronic infections, loss of the infected quarter, and culling of animals. Among the species of the genus, *Prototheca zopfii* is the most prevalent species causing clinical and subclinical mastitis. This species is characterized by two genotypes (1 and 2), of which genotype 2 is widely associated with mastitis and genotype 1 is considered non-pathogenic. *P. blaschkeae* (the previous biotype III of *P. zopfii*) is considered pathogenic but not as prevalent as *P. zopfii*. Thus, the objective of this study was to use molecular methods to identify *Prototheca* species and genotypes isolated from clinical mastitis cases. Twenty isolates were obtained from clinical cases occurred on 3 dairy herds located in São Paulo state, Brazil. The protocol described by McCullough (2000), commonly used for yeasts, was used to obtain DNA. Extraction and quantification of DNA was performed by use of a spectrophotometer. For diagnosis of species and genotypes, Polymerase Chain Reaction (PCR) was performed using the primers Proto18-4f, Proto18-4r, PZGT 1/r, PZGT 2/r, PZGT 3/r, PZGT 3-IK/f and PZGT 3-IK/r, followed by electrophoretic run on agarose gel. All isolates were identified as *P. zopfii* genotype 2. These results agree with previous findings indicating this genotype as the main causative agent of *Prototheca* mastitis. The fact that 17 isolates of the same species and genotype were isolated from the same farm suggests adaptation within the herd and possible contagious transmission among cows. The higher incidence of *P. zopfii* genotype 2 may be related to genetic characteristics that determine virulence mechanisms that facilitate survival in the environment and the mammary gland. Molecular characterization of the pathogen can be an important tool to understand the epidemiology of the disease and improve the efficiency of mastitis control programs.

**Key-words:** bovine mastitis, algae, *Prototheca* spp, genotypes

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