

**TITLE:** DENATURING GRADIENT GEL ELECTROPHORESIS TECHNIQUE FOR DETECTION GRAM NEGATIVE BACTERIA IN SOILS OF DAIRY FARMS OF SOUTH FLUMINENSE REGION – RJ

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**ABSTRACT:**

Bovine mastitis is a complex disease that may have different causes, degrees of intensity, duration variations and consequences on the quantity and quality of the milk produced. Environmental mastitis, whose infection occurs in the interval between the milking processes, inside the pickets, is considered to be one of the main problems found in animal production, with severe repercussions in the domestic market and internal milk supply. *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterobacter cloacae* are Gram negative bacteria commonly found in manure, feces, urine and organic beds contaminated by animal excretions. These bacteria are important agents that lead to the infectious process, culminating with the appearance of mastitis. The objective of the present work was detect Gram negative bacteria present in the soil that may be involved in the etiology of bovine mastitis, in a dairy farm in the Barra do Pirai, South Fluminense region-RJ. Four soil samples were collected at different seasons of the year (spring and winter of 2015, summer and autumn of 2016), considering the most recently pickets used by cattle, totalizing 16 samples. After DNA extraction directly from the soils, amplification of the 16S gene from the bacterial rDNA was performed by PCR (Polymerase Chain Reaction) and the amplification products were separated by DGGE (Denaturing Gradient Gel Electrophoresis). In all DGGE gels were added a molecular marker containing amplification products of five bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterobacter cloacae* and *Clostridium botulinum*. The presence all the Gram negative bacteria in the samples was observed, with emphasis on *E. coli* and *Pseudomonas* spp. that were detected in 100%. Species of *Proteus* and *Enterobacter* were detected in 88.9% and 72.2% of the samples, respectively. Considering that the pathogenic bacteria are found in great quantity in the pickets that are places where the cows lay and spend most of the day, a range of alternative strategies for control and prevention are important, aiming to reduce the cases of mastitis in the farm.

**Keywords:** DGGE marker, Enterobacteriaceae, environmental mastitis, PCR (Polymerase Chain Reaction)

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