

TITLE: INTERACTION BETWEEN *Mycobacterium avium* subsp. *paratuberculosis* AND BACTERIUM CAUSING BOVINE MASTITIS, *Staphylococcus aureus* AND *Streptococcus agalactiae*, IN BOVINE MAMMARY GLAND EPITHELIAL CELL LINE- MAC-T

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ABSTRACT:

Although it is known that MAP, the etiological agent of paratuberculosis, is not directly related to mastitis, this bacterium is eliminated in milk, considered as the second route of elimination. In this way, the objective of this research was to evaluate the influence of MAP on coculture with the main bacteria causing bovine mastitis, *Staphylococcus aureus* and *Streptococcus agalactiae*, in mammary epithelial cells (MAC-T) at different times. Bacterial internalization, colony counts, molecular assays and viability evaluation of MAC-Ts cells were performed. In the internalization test between MAP and *S. aureus*, there was a 30 minutes time difference between MAC + *S. aureus* and MAC-T + MAP + *S. aureus* treatments. The MAC-T + *S. aureus* treatment showed significant differences between the 10 and 120 minutes ($p = 0.001$), and between 30 and 120 minutes ($p = 0.048$). The MAC-T + MAP + *S. aureus* treatment showed a difference between 10 minutes and 30 minutes ($p = 0.042$), and between 10 and 120 minutes ($p < 0.0001$). When the co-infection was performed with *S. agalactiae*, the trials showed no significant difference between the treatments. The treatment MAC-T + *S. agalactiae*, showed significant differences between the times 10 and 120 minutes ($p < 0.0001$), and between the times 30 minutes and 120 minutes ($p = 0.030$). The MAC-T + MAP + *S. agalactiae* treatment showed a difference between 10 and 120 minutes ($p < 0.0001$) and between 30 and 120 minutes ($p = 0.029$). The cell viability of MAC-T at all times of the internalization assays was verified by the test 3 - [4,5-dimethyl-thiazol-2-yl] -2,5-diphenyl-tetrazolium bromidetest (MTT), concluding that the internalization process was not influenced by the death of the MAC-T cells but by the presence of MAP, *S. aureus* and *S. agalactiae*. These results confirm the existence of the interaction between MAP and the classical agents of bovine mastitis, *S. aureus* and *S. agalactiae*, favoring the internalization of *S. aureus* and not influencing the invasion of *S. agalactiae* during the internalization tests in MAC-T cells, performed in the time worked, and may contribute to the worsening of mastitis and the permanence of these agents in the interior of mammary epithelial cells, which could result in compromised mastitis treatment.

Keywords: co-infection; MAC-T; MAP; paratuberculosis; qPCR.

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