

Molecular detection of *Mollicutes* and *Mycoplasma* spp. in milk samples, with absence of growth in conventional microbiological isolation and from bovines with mastitis

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Summary

This Research Paper addresses the hypothesis that bacteria of the genus *Mycoplasma* are present in mastitis milk samples that showed absence of bacteriological growth in conventional isolation. Mastitis is the main health challenge present in dairy farms, being highly prevalent and leading to significant economic losses for the sector. The control of mastitis is based on the diagnosis of the etiologic agent. Among the agents responsible for outbreaks of the disease, the genus *Mycoplasma* stands out, which needs specific diagnostic techniques for its detection. In Santa Catarina, data on the presence of this microorganism in milk are not available. In this regard, the study aiming to detect, through a PCR assay, the presence of bacteria from *Mollicutes* class and *Mycoplasma* genus, in milk samples negative to conventional microbiological isolation from cows with mastitis. For this purpose, a total of 187 milk samples of mastitis cases from dairy herds of the State of Santa Catarina were used. The samples were submitted to DNA extraction protocols using the phenol-chloroform method, thereafter the PCR was performed to identify the *Mollicutes* class. The positive samples were submitted to a new PCR reaction to the genus *Mycoplasma*. Subsequently, DNA fragments obtained in the genus-specific PCR amplification were cloned, the recombinant clones were sequenced and the DNA sequences obtained were subjected to homology analysis. Of the analyzed samples, 1.6% were positive for *Mollicutes* and 1.1% presented amplification for the genus-specific PCR, but the sequencing did not verify homology with the genus *Mycoplasma*. Therefore, the presence of this bacterial class in mastitic milk samples in Santa Catarina state was reported, allowing the inclusion of these microorganisms in the etiological suspicion of mastitis.