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Detection of Mollicutes species from Brazilian dairy cattle by molecular techniques

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Mollicutes are the smallest known free-living and self-replicating forms of life. In cattle, *Mycoplasma* spp. and *Ureaplasma* spp. are the most important genera related to bovine mycoplasmosis, since they are associated to illness such as mastitis and reproductive failures. Clinical outbreaks of mycoplasmosis are reported, nevertheless there are few publications about infected dairy herds without clinical signs of disease or occurring under subclinical conditions. The aim of this study was to investigate the presence of Mollicutes species by molecular identification in milk and vaginal swab samples from dairy cattle from Minas Gerais, which is an important dairy basin in Brazil. Milk and vaginal swab samples were collected from 20 dairy cows randomly selected. DNA extraction was executed by a phenol-chloroform-isoamyl alcohol protocol for milk samples and by a phenol-chloroform protocol for vaginal swab samples. PCR for Mollicutes detection based on the amplification of 16S rRNA sequences was performed with a 270 bp amplicon. Positive samples were after purified using Illustra GFX PCR DNA and Gel Band Purification Kit/GE™. DNA sequencing of 16S rRNA was carried out with chain-termination dideoxiribonucleotides using Big Dye Terminator (Applied Biosystems, Foster City, USA). The sequences were edited using SeqMan II software (Lasergene, DNASTar) and compared to those available on GenBank® using Nucleotide BLAST®. Ten percent (2/20) of milk samples and 100% (20/20) of vaginal swab samples were positive in PCR for Mollicutes detection. Sequencing of 16S rRNA from milk samples resulted in an identity of 98% to *Ureaplasma diversum* and 97% to *Mycoplasma bovis*. From 20 vaginal swab samples, 25% (5/20) resulted in a 98% match to *U. diversum* and 5% (1/20) presented an identity of 95% to *M. bovis*. Other swab samples resulted in a low (< 90%) match to Mollicutes species or did not show any identity to Mollicutes. Genomic sequencing was able to identify Mollicutes species from milk and vaginal swab samples. Further studies are necessary to evaluate strategies of genomic sequencing, such as using larger amplicons (>270 bp). Notwithstanding, our results highlight that Mollicutes species are present in cattle from an important Brazilian state in dairy production.