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MOLECULAR IDENTIFICATION OF MOLLICUTES INFECTION AND ASSOCIATED RISK FACTORS IN DAIRY CATTLE UNDER TROPICAL CONDITIONS

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RESUMO

Diseases associated with bacteria in Class Mollicutes can cause decreased milk production and reproductive disorders in cattle, contributing to economic damage and compromising animal welfare. This study aimed to investigate the presence of Mollicutes and *Ureaplasma diversum* microorganisms in the reproductive tract of dairy cows and to determine the risk factors associated with infection, as well as to identify strains of circulating ureaplasmas in herds from Minas Gerais, Rio de Janeiro and São Paulo states. Vaginal swab samples from 392 lactating cows were subjected to generic polymerase chain reaction (PCR) for the detection of Mollicutes and specific PCR for the identification of *U. diversum*. Concomitantly, an epidemiological questionnaire was applied to analyze the risk factors (OR) associated with infection. Wald test ($p < 0,10$) was used for the selection of variables to compose the multiple model. For the regression analysis it was used the least absolute shrinkage and selection operator (LASSO), using R Core Team (2021). A phylogram was generated using Geneious Prime® and the neighbor-joining tree method was performed with the genetic distance model Tamura-Nei and the bootstrapping of 10,000 replications. The geographical coordinates of the municipalities where the herds were located were used to perform the phylogeographic analysis employing MicroReact®. Positive samples for *U. diversum* were sequenced from the intergenic region 16S-23S rRNA. Regarding to PCR results, 45.4% (178/392) and 21.7% (85/392) of the samples were positive for generic and specific PCR (*U. diversum*), respectively. Regarding risk factors, number of animals per herd over 150 (OR 11.78, 95% CI: 4.806- 33.565) and repeat breeding (OR 3.9, 95% CI: 2.363-6.438) were associated with Mollicutes infection, while herd size greater than 150 animals was associated with *U. diversum* infection (OR 3.3, 95% CI: 1.225-11.379). Regarding the genomic sequencing analysis, were observed two prominent clusters including strains from Minas Gerais, São Paulo and Rio de Janeiro states. The frequency of the agents was high and different strains of *U. diversum* are spread throughout the studied states, probably due to the great animal movement network. In

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addition, the herd size was an important risk factor to occurrence of Mollicutes.

PALAVRAS-CHAVE: Mollicutes, bovine, risk factors, PCR, 16-23S rRNA, molecular epidemiology

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