TITLE: VALIDATION OF REAL-TIME PCR (qPCR) TECHNIQUE FOR DETECTION OF *Mycobacterium bovis* AND *Brucella abortus* IN RAW MILK SAMPLES**.**

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ABSTRACT:
Tuberculosis and bovine brucellosis are infectious diseases of global spread caused by the etiological agents *Mycobacterium bovis* and *Brucella abortus*, respectively, which cause substantial economic losses and can be transmitted to humans through direct contact with contaminated animals and ingestion of raw milk and dairy products. In Brazil, there is the National Program for the Control and Eradication of Brucellosis and Tuberculosis, which aims to reduce the prevalence and incidence of genotypes in cattle and buffaloes for eradication, and establish the diagnosis, regulating the control measures of these zoonosis. The official methods for diagnosis of brucellosis and tuberculosis in the living animal have variable sensitivity and specificity, are laborious and time consuming. In order to increase the efficacy of brucellosis and tuberculosis control, rapid and accurate methods are necessary to act as an auxiliary tool to the *in vivo* diagnosis of these diseases without the need of invasive procedures. To ensure the credibility and reliability of new diagnostic methods, they must be validated. In the present study, the validation of a real-time polymerase chain reaction (qPCR) for the detection of *M. bovis* and *B. abortus* in artificially contaminated raw milk samples was performed using analytical performance (analytical sensitivity and specificity), repeatability, internal reproducibility and robustness. Initially five DNA extraction methodologies were tested and the ones that presented the best results were the commercial kits “DNeasy Mericon Food Kit – Qiagen” and “Maxwell® 16 Tissue DNA Purification Kit – Promega”. The limits of detection obtained in the qPCR validated in this study were 2.3 pg of *M. bovis* DNA and 20.7 fg of *B. abortus* DNA. The repeatability and reproducibility associated with the robustness presented in this study indicate that the evaluated methods can be used as an auxiliary tool to the *in vivo* official diagnosis of bovine tuberculosis and brucellosis, after being tested in naturally infected animals.

Keywords: bacterium; bovine; molecular technique; zoonosis

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