TITLE: VALIDATION OF A MOLECULAR TEST FOR BOVINE TUBERCULOSIS *POSTMORTEM* DIAGNOSIS

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ABSTRACT:

Bovine tuberculosis (bTB) is a zoonotic disease which impacts on animal health and economy. For bTB *postmortem* diagnosis, the etiologic agent *Mycobacterium bovis* needs to be isolated from biological samples with suggestive lesions. However, this procedure is time-consuming and requires level 3 biosafety installations. Therefore, methods such as Polymerase Chain Reaction (PCR) could provide safer and faster results. The main objective of this study was to validate a molecular method for the *postmortem* bTB diagnosis. A total of 171 samples of bovine tissues were collected in official slaughterhouses of Rio Grande do Sul. The samples were submitted to bTB diagnosis by PCR targeting *M. bovis*, histopathology and bacterial isolation. The tests' results were compared to the reference method (bacterial isolation) by Stata 12.0 software. PCR detection limits with and without biological matrix were evaluated by standard curve with known amounts of *M. bovis* DNA molecules. PCR was able to detect 10^2 and 10^3 *M. bovis* DNA molecules in the absence and presence of biological matrix, respectively. The sensitivities of the alternative methods were low (43.6% - PCR; 70.8% - histopathology), and the specificities were superior (81.4% - PCR; 81.2% - histopathology). The combination of histopathology and PCR resulted in increased sensitivity indicating that these could be used as screening tests. As the PCR was not accurate, it was investigated whether the lesion stages would influence the ability of *Mycobacterium* detection through this method. The results showed that in advanced lesion stages, the sensitivity of PCR was higher (43.8%), compared to the initial stages (28.0%), although the number of evaluated samples in the early stages was lower. The low PCR sensitivity can be explained by the low bacterial loads in the samples, DNA extraction method or due to genetic variability of the bacteria. This study demonstrated that neither histopathology nor PCR can be used in replacement of *Mycobacterium* spp. isolation, but both could be applied together as a screening method, in which positive concordant results would be considered final diagnosis (specificity of 94%) and discordant results would lead the samples to bacterial isolation. These results evidence the importance of validation for molecular diagnosis. Future studies on alternative DNA extraction methods must be performed in search of a better test performance.

Keywords: *Mycobacterium* spp., bovine tuberculosis, molecular diagnosis, validation

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