RAPID DETECTION OF TUBERCULOSIS IN BEEF CARCASSES

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I. INTRODUCTION

Bovine tuberculosis (TB) is a chronic bacterial disease caused by microorganisms from the *Mycobacterium tuberculosis* complex, mainly *M. bovis*. Many batches of cattle are disqualified for export due to suspicion of TB established during visual, ante- and post-mortem inspections, carried out during the slaughter of cattle, by the official Brazilian Veterinary Inspection Service (SIF) without proper laboratory confirmation. Therefore, disabling batches of animals for export based exclusively on visual observation is beyond the modern sense of inspection, economy, and sustainability. Fast, sensitive, and specific analytical techniques, without the need for laboratory structure and trained technical personnel, are available for the diagnosis of human tuberculosis and are approved for use by government health agencies (Brasil, 2022). The objective of the present study was to apply the qPCR technique, using the GeneXpert equipment and the MTB/RIF kit (GXMTB), on samples collected directly from lesions on carcasses suspected of TB defined by the SIF. The results for detection of *M. tuberculosis* Complex agents, obtained from this new sampling matrix, were compared with those from microbiological culture (MC), considered the gold standard method.

II. MATERIALS AND METHODS

100 samples were analyzed, 94 originating from lesions suspected of TB and six from lesions considered non-specific by the Federal Inspection. Initially, the samples were collected with a swab from the injured organ/tissue and placed in a conservative solution provided in the GXMTB kit, immediately applying the protocol indicated by the manufacturer (Cepheid®, 2020). Samples of 1g were taken from the same injured organs/tissues, placed in an isothermal box containing ice and sent to the laboratory of the Department of Preventive Veterinary Medicine and Animal Health at FMVZ-USP, where they were inoculated in Lowenstein-Jensen and Stonebrink culture media. DNA was extracted from typical colonies and conventional PCR was applied to differentiate *Mycobacterium* spp., *M. tuberculosis* and *M. bovis*. The lesions were also subjected to histopathological and cytological analysis. The results obtained through the GXMTB protocol and MC were evaluated using the McNemar and Kappa statistical tests to determine agreement and replicability between them.

III. RESULTS AND DISCUSSION

The results of the analysis with the GXMTB kit were obtained, on average, 77 minutes after sampling. When comparing the results from GXMTB and MC methods by the McNemar test, no

disagreement was observed between them (p=1). However, there was disagreement between the SIF and MC results (p < 0.0001) and between SIF and GXMTB (p = 0.0034). The application of the Kappa test indicated that the replicability was considered as “GOOD” when comparing the results between the GXMTB and the MC (p < 0.0001), and replicability was considered “BAD” when comparing the other analytical methods investigated (p < 0.0001). Histopathology and cytology confirmed lesions characteristic of TB, as well as the presence of acid-alcohol resistant bacilli. All isolates were confirmed by conventional PCR as *M. bovis* and all of them didn’t show Rifampicin resistance.

Table 1 – Results of 100 samples processed after veterinary inspection showing agreement and disagreement between the three methods applied.

Results	+ +	+ -	- +	- -	Total
GXMTB / SIF	82	1	12	5	100
GXMTB / MC	77	6	6	11	100
SIF / MC	81	13	2	4	100

Handling the samples, as well as performing the analyzes with the GXMTB kit, were extremely fast and easy, providing robust results without the need for specialized laboratories or highly trained personnel, especially when compared to traditional methods that take up to 100 days. The short time required -less than 90 minutes- to obtain results allows confirmation of suspected TB even when the meat has not completed sanitary maturation, providing an extremely useful tool for determining destination markets.

IV. CONCLUSION

The GXMTB analytical method showed agreement/replicability with the MC (Gold Standard) for detection and identification of *M. bovis* and was efficient when applied from a new sample matrix. It was extremely fast and easy to handle, providing robust results without the need for specialized laboratories or highly trained personnel, especially when compared to traditional methods that take up to 100 days. Therefore, it can be used as an auxiliary method in the immediate diagnosis of TB. The precision and speed in obtaining results by GXMTB allowed TB confirmation when the carcass has not yet completed its sanitary maturation, thus enabling safety decisions regarding the destination of the carcasses and the qualification/disqualification of the animals’ batch with exportation potential. Only *M. bovis* was detected and none of them showed Rifampicin resistance.

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