

**Direct detection of *Mycobacterium* sp in bovine and bubaline tissues by PCR** - Araújo C.P.<sup>1</sup>, Osório A.L.A.R.<sup>1</sup>, Jorge K.S.G.<sup>1</sup>, Ramos C.A.N.<sup>2</sup>, Filho A.F.S.<sup>1</sup>, Vidal C.E.S.<sup>3</sup>, Roxo E.<sup>4</sup>, Rocha A.<sup>5</sup>, Fonseca Júnior A.A.<sup>6</sup>, Silva M.R.<sup>7</sup>, Araújo F.R.<sup>8\*</sup>

1 - Programa de pós-graduação em Ciência Animal FAMEZ/UFMS, Campo Grande, Mato Grosso do Sul, Brazil

2 - Bolsista DTI- CNPq. Mato Grosso do Sul, Brazil

3 - Aluno de doutorado PPGMV-UFSM, Rio Grande do Sul, Brazil

4 - Pesquisadora científica do Instituto Biológico de São Paulo, Brazil

5 - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

6 - Laboratório Nacional Agropecuário de Minas Gerais, Brazil

7 - Pesquisador da Embrapa Gado de Leite, MG, Brazil

8 - Pesquisador da Embrapa Gado de Corte

\*poster presenter: [flabio@cnpqg.embrapa.br](mailto:flabio@cnpqg.embrapa.br) (F.R. Araújo)

In this study, a two-step PCR system was developed to detect *Mycobacterium tuberculosis* complex or *Mycobacterium bovis* directly from cattle tissues. The first reaction was conventional PCR, followed by a second real-time PCR with internal primers and 6FAM/MGBNFQ probe, both reactions for *rv2807* (*M. tuberculosis* complex) or *mmp6* (*M. bovis*). Among the 92 DNA samples isolated from cultures of *M. bovis*, 91 (98.9%) were positive for *rv2807* and 89 (96.7%) for *mmp6*. All of the 170 DNA samples from culture of *M. tuberculosis* were positive for *rv2807*. Primers for *rv2807* and *mmp6* did not amplify DNA of *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Corynebacterium pseudotuberculosis*, *Haemophilus influenza*, *M. avium*, *M. fortuitum*, *M. goodii*, *M. kansasii*, *Nocardia asteroides* or *Rhodococcus equi*. Primers for *mmp6* did not amplify DNA of *M. tuberculosis* H37Rv, but 4 false positive reactions were detected among the 170 culture samples of *M. tuberculosis* (2.4%). With regards to the 66 tissue samples from cattle with positive intradermal skin tests (IST), among the 40 samples with lesions compatible with tuberculosis (LCT), 34 (85%) were positive for *rv2807*, 31 (77.5%) for *mmp6* and 36 (90%) for bacilloscopy (culture+/fast acid bacilli+). Among the 26 samples with no visible lesions (NVL), 17 (65.3%) were positive for *rv2807*, 12 (46.2%) for *mmp6* and 13 (50%) for bacilloscopy. No amplifications were detected with PCR for *rv2807* neither for *mmp6* and tissues from 44 IST negative cattle. In other experiment, 30 LCT were cultured and stored for up to 4 years before PCR processing, resulting in 14 (46.6%) reactions for *rv2807*, 9 (30%) for *mmp6* and 13 (43.3%) for bacilloscopy. Also, among the 20 water buffalo and four bovine tissue samples with LCT, transported in poor conditions (high temperatures) and partial decomposition, 15 (62.5%) were positive for *rv2807* and 13 (54.1%) for *mmp6*.

**Key-words:** bubaline and bovine tuberculosis, post-mortem diagnosis, PCR

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