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Comparative evaluation of LAMP and Nested-PCR for the diagnosis of bovine paratuberculosis

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ABSTRACT

Introduction: *Mycobacterium avium* subsp. *paratuberculosis* causes paratuberculosis (Johne's disease), a systemic infection and chronic inflammation of the intestine that affects many species, including bovine. Infection is widespread in livestock, and human populations are exposed. A possible association between MAP infection and Crohn's disease in humans has been also described. Effective control of paratuberculosis has hampered due to lake of rapid and accurate diagnostic test. Range of diagnostic tests is available, but all have inborn limitations. The present study was designed to develop a loop-mediated isothermal amplification (LAMP) assay for the rapid and simple detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

Materials and methods: Six primers were specially designed for recognizing eight distinct sequence of insertion sequence 900 (IS900). To determine the sensitivity of the LAMP assay, 10-fold serial dilutions were made from 431 ng/μl MAP stock solution and compared with Nested-PCR results obtained using similar templates at identical concentrations. Detection limit of the LAMP was defined as the last positive dilution and the reactions were performed four times to examine the reproducibility of the test. The specificity of the assays were evaluated by testing three Gram-positive bacteria including *Mycobacterium bovis* AN5, *Mycobacterium tuberculosis* DT and *Mycobacterium avium avium*.

Results: Sensitivity of this assay for detection of DNA of MAP was 4 fg/μl and the specificity was 100%. This assay successfully detected MAP not only in the bacterial cultures but also in clinical fecal samples and the specificity of both PCR was 100%. This LAMP method is performed under isothermal conditions and no special apparatus is needed. In addition, its reactivity is directly observed with the naked eye without electrophoresis either as

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turbidity or in the form of a color change when SYBR Green 1, a fluorescent dsDNA intercalating dye, is employed.

Conclusions: This assay is rapid which requires nearly 1 h for detection of MAP, low in cost and simple to perform, sensitive and practical tool for the detection of MAP and will be useful in facilitating the early diagnosis of paratuberculosis (Johne's disease) caused by the organism.