

Molecular Detection of Mycoplasma Gallisepticum and Mycoplasma Synoviae using SYBR Green based Quantitative PCR Assay in Selected Live Bird Markets Located in Western, Central and North Western Province in Sri Lanka

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Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are one of the key avian pathogens that exclusively infect the poultry industry globally. Infected chickens exhibit a wide range of respiratory symptoms and lead to considerable economic losses due to poor productivity, reduced egg production, carcass condemnation and decreased feed efficiency, all of which increase production cost. Rapid and accurate diagnostic methods are essential for preventing and controlling Mycoplasmosis more effectively. This study aimed to develop and optimize a convenient and rapid SYBR Green-based quantitative PCR (qPCR) assay for accurate and efficient detection of MG and MS in poultry. Previously published primers targeting the *16s rRNA* gene were utilized to detect the presence of *Mycoplasma* spp. Additionally, species specific primers targeting the *mgc2* gene and *vlhA* gene were used to detect MG and MS, respectively. Melt curve analysis were deployed to assess the specificity and accuracy of the developed assays. The melting temperatures of the amplicons for *Mycoplasma*, MG and MS were determined to be 82°C, 77.5°C and 79 °C, respectively and detected as a single melt peak without cross reaction with other non-targeted pathogenic agents. Developed assays were used to detect the prevalence of DNA originated from MG, and MS in selected poultry live markets located at the Central, Western, and Northwestern provinces in Sri Lanka. Out of 300 tracheal swabs collected from live bird markets, revealed 36.66% (110/300) *Mycoplasma* spp. Prevalence level across the study areas. Out of the positive samples, MG was present in 81.81% (90/110) whilst MS present only in 18.18% (20/110) indicating MG infections were common in selected live bird markets. The developed assays can be utilized for rapid detection of Mycoplasmosis in poultry that could assist controlling the disease at early stages. Further, reported prevalence of Mycoplasmosis in selected live bird markets in Sri Lanka warrant further studies and regulations to control the disease.

Keywords: *Mycoplasma Gallisepticum* (MG), *Mycoplasma Synoviae* (MS), Quantitative PCR, SYBR Green, Prevalence