Real Time PCR Quantification and Differentiation of both Challenge and Vaccinal Mycoplasma gallisepticum trains Used in Vaccine Quality Control.

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ABSTRACT
Mycoplasma gallisepticum is an economically important pathogen of poultry worldwide, causing chronic respiratory disease in chickens and turkeys. Vaccination of poultry with Mycoplasma gallisepticum live vaccines is an approach to reduce susceptibility to infection and to prevent economic losses. The goal of this study was to develop an alternative method for evaluation of live and killed vaccine using quantitative differential real time PCR (rt-PCR) assay. Real time PCR assay was implemented for titration and identification of three types of Mycoplasma gallisepticum (F, ts-11 and field strain). Three groups of chicks were vaccinated by using F-strain, ts-11 and killed vaccine and the forth group was considered control. Challenge test was applied by using Mycoplasma gallisepticum field strain (10^8 CFU) at three weeks post vaccination. Antibody ELISA titers against Mycoplasma gallisepticum were 319, 259 and 1009 for F, ts-11 and killed vaccine respectively at 3 weeks post vaccination. The protection rates were 81.5%, 74%, and 66.6% for F-strain, ts-11 and killed vaccine respectively that was determined by air sac lesion scour. Using quantitative differential rt-PCR for necropsied birds at 5 days post challenge 7 days post challenge and 14 days post challenge demonstrated that the F-strain vaccine had ability to prevent shedding of field strain at 14 days post challenge mean while the ts-11 and killed vaccine decreased shedding of field strain from 10^8.1 and 10^8.6 to 10^5.1 and 10^5.8 CFU respectively at 14 days post challenge. In this study, rt-PCR had ability to identify and quantify of two types of vaccines (F and ts-11) and field strain.

Keywords: Mycoplasma, rt-PCR, Vaccine, Poultry