Real Time PCR Quantification and Differentiation of both Challenge and Vaccinal Mycoplasma gallisepticums 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, t-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 to 10^5.1 and 10^8.6 to 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
ABSTRACT

Potential harmful threat to human health has led to a need to find safe alternatives for the use of antibiotics. The young poultry may be infected with several bacterial diseases including Salmonella spp and Escherichia coli. In addition, the emergence of antimicrobial resistance to antibiotics is a serious problem for the poultry industry.

In this study, we have investigated the effect of essential oils extracted from five different herbal plants against Salmonella and Escherichia coli. The effects of these essential oils were compared with tetracycline. The results showed that the essential oils of Carum copticum had the highest antibacterial properties. The maximum inhibition zone in diameter against Salmonella was 26.7 mm, while against Escherichia coli, it was 22.5 mm. These results suggest that Carum copticum essential oil might be used as an alternative to antibiotics for treating infections caused by these bacteria in poultry industry.

The experiments were conducted on commercial chicks during the winter months (December and January). Day old commercial meat type broiler chicks (273) were procured from a reputed source. Cold conditioning (20°C to 80°C) at third and fourth day of age for 3-4 hours was provided to all treatment groups. A biological trial was conducted on commercial chicks during the winter months (December and January) to determine the effects of different herbal plants on the performance of broiler chickens. The broiler chicks received daily 200 ppm lithium carbonate in their water, for 20 days and control group received water without lithium. At the end of second week, the chicks were individually weighed, distributed into 7 treatment groups of 3 replicates with 13 chicks in each replicate. Cold challenge @ 20°C to 80°C for 8 hours was provided from third week of age to sixth week of age. There was no significant difference in the performance of the birds in the treatment groups T1 and T5. There was no significant difference in the performance of the birds in the treatment groups T7.

In conclusion, the results of this study showed that the essential oils of Carum copticum have a stronger antibacterial effect than tetracycline. So, after the complementary studies, some herbal plants can be suggested as alternatives to antibiotics for treating infections caused by these bacteria in poultry industry.