Study of Interaction Between AviPro® Mycoplasma gallisepticum (MGF) and Pulmotil®-AC in Broiler Breeder Pullets

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Abstract
This study aimed at the determination of the optimal time interval between the vaccination with a live Mycoplasma gallisepticum vaccine AviPro® MGF and the administration of the antibiotic Pulmotil® AC (PAC) to broiler breeder pullets while preserving the vaccine efficacy. A total of 108 sixteen-weeks-old breeder pullets of the ROSS 308 strain were subdivided equally into 6 groups. Pullets of group 1 remained unvaccinated and untreated with PAC. Birds of groups 3, 4, 5 and 6 were vaccinated with AviPro® MGF at 16 weeks of age and treated with PAC at 3, 7, 14, and 21 days post-vaccination respectively. Group 2 was kept as the vaccinated non-treated group. The pullets were tested for the presence of MGF strain in the trachea at different days after PAC-treatment completion. All PAC-treated groups showed tracheal MG recolonization after the treatment was discontinued. The percentage of positive tracheal MG swab cultures was consistently higher in group 6, reaching a plateau at 14 days post PAC treatment (100%, P<0.05). qPCR implied-tracheal MG counts indicated better recolonization efficiency for birds of groups 5 and 6 reaching up to 2322 ± 103 and 2839 ± 103 cfu/ml of broth, respectively, at 35 days post PAC treatment. Moreover, Group 6 showed the significantly highest titer to MG, recording a value of 2160, followed by the vaccinated untreated Group 2 (1128). For a successful application of live MG vaccine/antibiotics combination, it is recommended to delay PAC treatment 21 days after the vaccination of breeder pullets with AviPro® MGF.

Keywords: Mycoplasma gallisepticum; AviPro MGF; Pulmotil AC; Pullets; qPCR; ELISA

Birds and housings
This experiment was approved by the Institutional Animal Care and use Committee (IACUC) of the American University of Beirut. It was conducted at the research facilities of the University in the Beqaa region where equipped poultry houses are available. A total of 108 sixteen-week-old pullets, of the Ross 308 strain, were equally subdivided into six groups of 18 birds each. Birds were given water ad libitum and feed as per the Breeder Manual recommendations provided by the breeding company. At arrival, swab samples were taken from the trachea of 10 birds to confirm that the birds were MG free using Frey’s culturing method and Polymerase Chain Reaction.

DNA sequencing of the MG vaccine F-strain
Upon receiving the AviPro® MGF, an aliquot of the vaccine suspension was subjected to DNA Extraction using the Qiagen DNA minikit (Qiagen GmbH, Hilden, Germany) and PCR amplification targeting a 267 bp fragment of the adhesin protein-coding gene (mgc2) [14]. The resulting amplicon was sequenced using the automated Sequencer 3100 Avant Genetic Analyzer- ABI PRISM Instrument (Applied Biosystems, Hitachi) to confirm the F strain identity of the experimental MG vaccine.

Evaluation of MG colonization in the trachea
A total of 10 individual tracheal swabs were taken at 6 different dates post-treatment namely 3, 7, 14, 21, 28 and 35 days. Swab rubbings were collected in 5 mL of Frey’s Broth which were then equally divided into two separate sterile tubes (2.5 mL/tube) and tested for the presence of MGF. The quantitation of MG colony forming units was performed using culture and real time PCR (qPCR) according to Grodio et al. as detailed below. Determination of the frequency of positive MG tracheal swab samples by Frey’s Broth culture: The first portion of tracheal rubbings in Frey’s broth (2.5 mL/tube; 10 samples per group) was incubated at 37°C for a period of one week. Samples were considered positive whenever the broth color turned orange, as a result of sugar fermentation and pH drop, within a range of three-seven days.

Conclusion
For a successful combination of live vaccine and antibiotics to be recommended, it is more preferable to separate PAC treatment 21 days after the administration of AviPro® MGF live vaccine to broiler breeder pullets. This will ensure efficient treatment of AviPro® MGF vaccinated birds with Pulmotil® AC while preserving the vaccine potency.

References
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