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Stability of Rehydrated *Mycoplasma gallisepticum* Vaccine Homogeneity over Time

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Abstract: Proper vaccine application is required to maximize the results of the vaccination, while maintenance of a homogenous vaccine solution is critical to obtain uniform results. This study was designed to analyze the need for continuous mixing of rehydrated *Mycoplasma gallisepticum* vaccine solution in order to maintain a homogeneous solution during vaccine application. Commercial F-strain vaccine (AviPro® MG F) was rehydrated and diluted in phosphate buffered saline in accordance with field practices. Dextrose was added to the solution to maintain *M. gallisepticum* viability without growth during the experiment. The vaccine solution was poured into columns and samples from the static solution were taken from 1, 25 and 50 cm above the base of the column. Samples were taken at 15, 30, 60 and 120 min and compared to a control that was mixed prior to sampling. The results indicate that no significant change in vaccine concentration occurred over the course of the experiment when comparing the mixed control to any of the samples. These results suggest that there is no need for continuous *M. gallisepticum* vaccine mixing if vaccine is applied within 2 h.

Key words: *Mycoplasma gallisepticum*, Vaccine, Homogeneity

INTRODUCTION

Mycoplasma gallisepticum is a member of class Mollicutes. The distinguishing features of this class include no cell wall and small size (Razin *et al.*, 1998). *M. gallisepticum* infects both domestic poultry species as well as wild avian species (Levisohn and Kleven, 2000). The results of infection can range from no clinical signs to airsacculitis, sinusitis and conjunctivitis in domestic poultry and wild avian species (Levisohn and Kleven, 2000). Infection can also result in carcass condemnation in meat-type birds (Evans *et al.*, 2005). The end result of *M. gallisepticum* infection is increased costs for poultry producers (Evans *et al.*, 2005).

Mycoplasma gallisepticum infection can be controlled through multiple means, including biosecurity and vaccination (Evans *et al.*, 2005). Antibiotics have been used to control *M. gallisepticum* infection; however, it is generally believed that antibiotic treatment only suppresses *M. gallisepticum* infection and infected birds remain carriers (Kleven, 2008; Ley, 2003). Biosecurity is used to prevent *M. gallisepticum* infection and it is effective in all-in/all-out poultry operations where meat type birds are raised (Evans *et al.*, 2005). In multi-aged layer operations, *M. gallisepticum* infection is endemic and the only economically viable means to control infection is through vaccination (Evans *et al.*, 2005). Currently, the only vaccines known to efficaciously protect against *M. gallisepticum* infection are “live” vaccines, including the F, 6/85 and ts-11 strains of *M. gallisepticum* (Evans *et al.*, 2005). The method of vaccine application has a dramatic effect on the overall success of the vaccination in a flock (Branton *et al.*, 2010).

During studies on proper application of the live *M. gallisepticum* vaccines it has been assumed that continuous mixing of the vaccine was required to maintain a homogenous vaccine solution. Two of the live *M. gallisepticum* vaccines (F and 6/85) are supplied in a lyophilized format and it is unknown how the lyophilization and rehydration process impact the ability of the vaccines to stay in solution. Many studies that have been performed to determine the optimal conditions for spray vaccination of these lyophilized vaccines used a vaccinator that continuously mixes the vaccine solution (Branton *et al.*, 2010). However, many field operations lack vaccinators with this option (Branton *et al.*, 2005; Branton *et al.*, 2010). In an effort to understand the requirement for continuous vaccine solution mixing on the homogeneity of the rehydrated vaccine solution, the change in rehydrated *M. gallisepticum* vaccine titer at different heights of a static solution was determined over time.

MATERIALS AND METHODS

Commercial F-strain *M. gallisepticum* (AviPro® MG F, Lohman Animal Health, Winslow, ME) was used in this study. Vaccine was rehydrated in phosphate buffered saline (PBS, Thermo Fisher Scientific, Waltham, MA) to which 0.3% (w/v) dextrose was added. Rehydrated vaccine was diluted in PBS+dextrose to a final

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concentration of one dose per 0.5 mL, which is within the dilution range used for vaccine spraying in field operations.

Diluted vaccine was placed in PVC pipe columns with rubber septum ports at 1, 25 and 50 cm above the bottom of the column. Sufficient volume was added above the 50 cm level to ensure that after removal of all samples, the level of vaccine would not drop below the septum at 50 cm. Samples (1 mL) were removed using 1 cc syringes and 23 gauge needles. Needles were inserted through the septum bevel down to prevent any settling of mycoplasma on the end of the needle that could artificially bias sample data and samples were withdrawn such that agitation of the static column was minimized. Samples were collected at 15, 30, 60 and 120 min from all three column heights of the static columns plus a separate control sample that was mixed prior to each sampling. At the time the columns were filled, duplicate samples were collected from the vaccine solution to determine the input concentration of *M. gallisepticum*.

Mycoplasma were quantitated by Color Change Unit (CCU) Assay as described previously (Leigh *et al.*, 2008). Final bacterial counts were calculated by the method of Reed and Munch (Reed and Muench, 1938). Results from each sample were divided by the average of the duplicate starting samples and the data were expressed as percent of the input concentration. Two trials of the experiment were performed with two columns being sampled in each trial, for a total of four data points at each sample location and time.

Samples were analyzed to ensure there was no trial by treatment effect. All results were combined based on sampling location and time. Significance of results was analyzed using the Repeated Measures ANOVA of SAS Analyst and were considered to be significant if $p \leq 0.05$ (SAS Institute, 2003).

RESULTS AND DISCUSSION

M. gallisepticum concentrations in the control group, as measured by CCU, were stable over the 120 min of the experiment. This is significantly different from previous research which demonstrated a decrease in *M. gallisepticum* viability over a 60 min experiment (Leigh *et al.*, 2008). Dextrose was added to the PBS as the presence of an energy source was hypothesized to prevent the decrease in viability over time (Leigh *et al.*, 2008). These results suggest that the addition of 0.3% dextrose (w/v) prevented the decrease in vaccine viability, at least over the 120 min of this study. The low concentration of dextrose was unlikely to have any additional impact on results beside increasing the duration of *M. gallisepticum* viability.

Prior to this work, the necessity of constant mixing of the vaccine to maintain a homogenous solution was unknown, but hypothesized to be required. As the rate of vaccine settling following rehydration was unknown, this work was performed to determine how the vaccine solution homogeneity changed based on hypothetical fluid levels within a vaccine reservoir over time. Samples taken at each time point following establishment of the static vaccine mixture in the column showed no significant difference in *M. gallisepticum* concentration at any height ($p>0.05$, Fig. 1). This suggests that rehydrated *M. gallisepticum* vaccine does not settle out of solution at a rate that would significantly influence it's concentration during vaccine application.

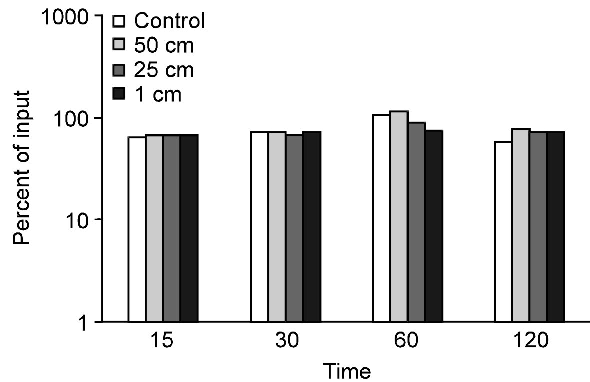

Fig. 1: *Mycoplasma gallisepticum* vaccine concentration at various heights over time in a static column. The heights are measured above the base of the column. The control group was mixed prior to sampling

These results demonstrate that continuous mixing of the vaccine solution during application is unnecessary for maintenance of a homogenous solution. However, as the vaccine is first rehydrated in the confines of the vaccine vial prior to mixing into a much larger volume for spray application, the ability to mix the complete volume of vaccine solution is necessary to ensure that a homogenous vaccine solution is obtained prior to application. The mixing or recirculating features present in some vaccination equipment (Branton *et al.*, 2005) would be advantageous to obtain a homogenous vaccine solution when preparing the solution, even if not required to maintain homogeneity of the solution.

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