Effect of Delivery Route on the Efficacy of a Liposomal Aspergillus Protein Vaccine against Pulmonary Aspergillosis in Dexamethasone Treated Chickens

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ABSTRACT

Pulmonary aspergillosis (PA) is an airway infection in poultry with limited treatment options. The efficacy of a liposomal Aspergillus protein vaccine (VesiVax®, Molecular Express Inc) was tested with a dexamethasone sodium phosphate (Dex SP) immunosuppressed PA model. SPF eggs were incubated for 21 days, hatched and moved to BSL2 rated cages. Birds were vaccinated (n = 8/group) on day 4 (d4) subcutaneously (sq) or mucosally (mu) and again d10 mu and d17 mu. Control birds received phosphate buffered saline. Animals were immunosuppressed intramuscularly with 8mg/kg Dex SP twice daily on d19-d24 and challenged mucosally d20 with *Aspergillus fumigatus* conidia. Lungs and tracheas were collected on d24 for fungal burden. Blood was also collected on d24 for an anti-spore agglutinating antibody assay. The vaccinated chickens were better protected against infection compared to non-vaccinated fungal challenged birds (327 vs 546 CFU/g lungs, P=0.005 with the sq, mu, mu regimen; 357 vs 546 CFU/g lungs, P=0.07 with the mu, mu, mu regimen). Tracheas of the vaccinated chickens showed better efficacy for birds given the mu, mu, mu regimen versus birds given the sq, mu, mu regimen (P = 0.037).

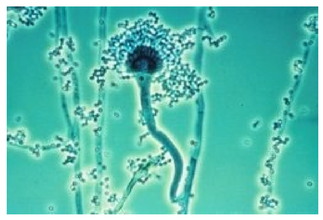
INTRODUCTION

Airway infections, are the second leading cause of poultry meat condemnation in the U.S.

- **Over 86 million US chickens condemned each year, which causes a significant loss to the $53 billion poultry industry [1].**

Aspergillus spp. causes airway infections

- **Aspergillus is a fungal species are commonly found in in the air, bedding, litter and feed of chicken houses.**
- **Poultry are susceptible to aspergillosis because avian respiratory tracts have a limited amount of ciliated columnar epithelium to aid in early fungal spore and hyphal clearance. [2,3].**
- **In addition, avian macrophages access to avian air sacs reducing their ability to phagocytize pathogens. Avian heterophils also lack the myeloperoxidase and oxidative mechanisms needed for killing fungi [2, 3].**



Aspergillus fumigatus Spores

Antifungal drug treatment for aspergillosis in poultry has had limited success

- **Treatment regimens involve a combination of topical and inhalation treatments which are logistically challenging [4].**
- **Given these limitations in antifungal drug treatment and the limitations of the poultry immune response to fungal clearance, the development of a preventative vaccine is needed.**

A candidate Aspergillus liposomal vaccine has been developed

- **Through a partnership amongst Dr. Jill Adler-Moore, Western University of Health Sciences, and Molecular Express, Inc. Using a unique, liposomal carrier system (VesiVax®).**
- **Based on these findings the next stage of development is to investigate routes of vaccine administration in the SPF chickens which is the focus of the present study.**

The aim of this study was to test and further optimize the vaccine protocol for commercial use in the poultry industry

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METHODS

Incubation & Hatching

- **Desired temperature and humidity over the three weeks of incubation:**

  Day 1-17: approximately 37.5°C, 50-60% humidity

  Day 18-21: approximately 37.5°C, 65-75% humidity

- **Brinsea OVA-Easy Advance Series II Incubator was set to tilt every 180 minutes and eggs were rotated by hand once a day 90 degrees until day 18.**
- **Eggs were candled and weighed after the 1st week and before the 3rd week. Heart rate was also taken on day 18, using the Avitronics Egg Buddy Digital Egg Heart Monitor.**

Egg Candler

Avitronics Egg Buddy Digital Egg Heart Monitor

Brinsea OVA-Easy Advance Series II Incubator

Biosafety Level 2 Isolation Cages

Vaccination

- **Birds (n = 8/group) vaccinated on d4 subcutaneously (SQ) and then mucosally (MU via eyes and nares) d10 and d17 or MU d4, d10, d17 with the vaccine using 5ug Aspf3 protein/dose and 5ug Aspf9 protein/dose with 5ug/dose of the adjuvant lipidated tucaresol.**

Immunosuppression (n=8/group)

- **Dexamethasone Sodium Phosphate (Dex s.p.) at 8 mg/kg given intramuscularly 2X daily on d20, d21, d22, d23 and d24 .**

Aspergillus Challenge

- **Eye and Nares (MU) Aspergillus Infection of Chickens**
- **(Birds challenged MU with A. fumigatus (ATCC 13073) on d21 with 5.4 X 10ex8 spores administered in 300 uL.**

Monitoring

- **Monitored after challenge 2X daily until d25 for signs of disease.**

Tissue Collection

- **Chickens euthanized on d25 (4 days post-fungal challenge), for tissue collection.**

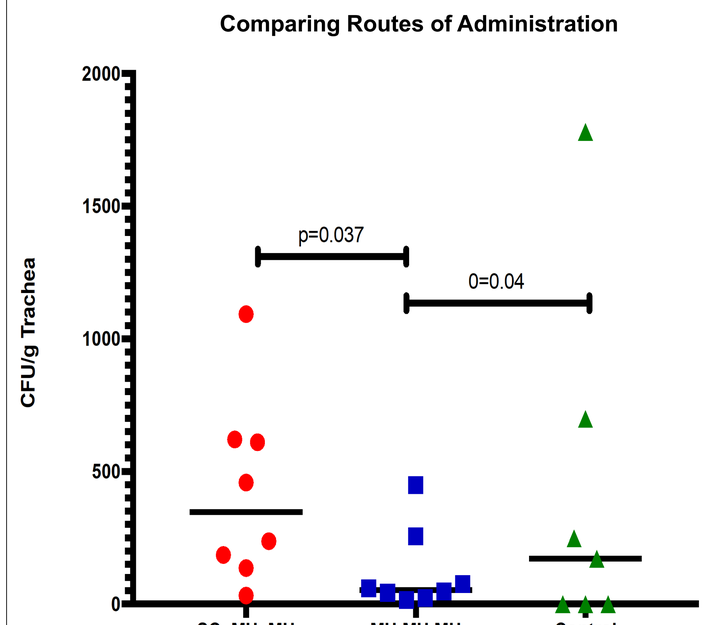
Analysis

- **Colony forming units (CFU) counted to obtain CFU/g lung or trachea. Serum was used for an agglutination titer in response to spores.**

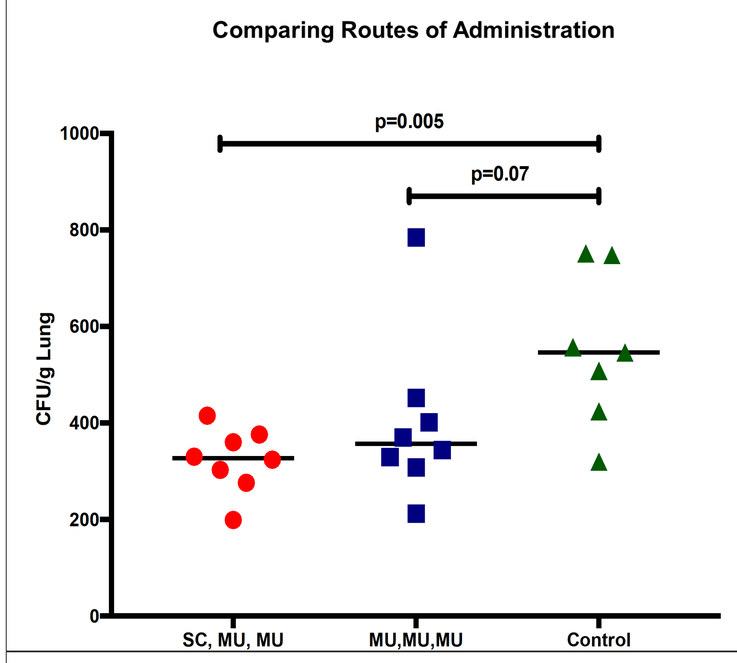
CONCLUSIONS

- **SC,MU,MU dosing and MU,MU,MU dosing with the Aspf3/Aspf9 liposomal Aspergillus protein vaccine decreased Aspergillus growth in the lungs following *Aspergillus fumigatus* pulmonary challenge.**
- **MU,MU,MU dosing provided comparable protection than the SC,MU,MU dosing regimen, based on producing significantly less Aspergillus growth in the tracheas and higher anti-spore antibody titers following Aspergillus challenge.**
- **Further optimization of the vaccine will be done by testing the effect of reducing the number of MU doses that have to be given to elicit significant protection.**

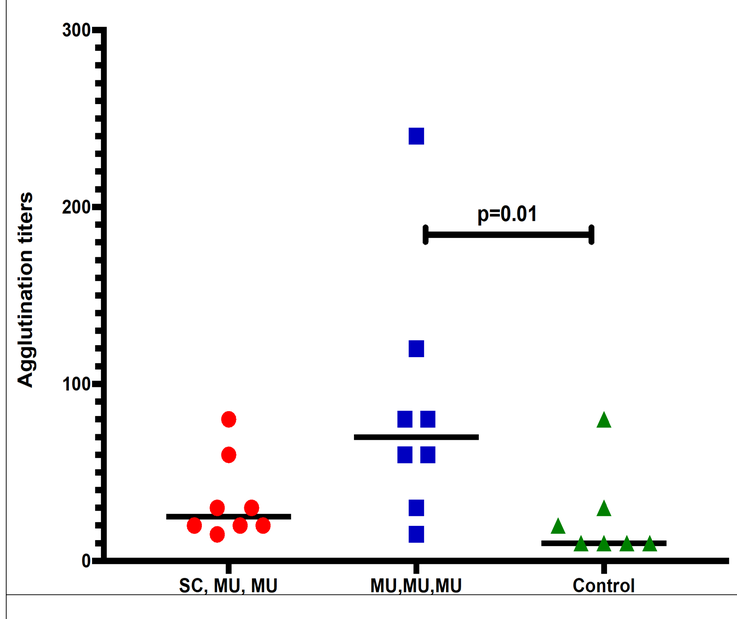
RESULTS



Chickens vaccinated MU, MU, MU with Aspf3/Aspf9/LT1 liposome vaccines were significantly better protected against infection, with lower CFU/g trachea versus non-vaccinated (control) birds (53 vs 171, P= 0.04) and birds given Aspf3/Aspf9/LT1 liposome vaccine (53 vs 347, P=0.037) (Two-tailed Mann Whitney t-Test)



Chickens vaccinated SC, MU, MU with Aspf3/Aspf9/LT1 liposome vaccines were significantly protected against infection with lower CFU/g lungs versus non-vaccinated (control) birds (327 vs 546,respectively, P=0.005). Chickens vaccinated IN, IN, IN trended to lower CFU/g lungs versus non-vaccinated chickens (357 vs 546; respectively, P=0.07) (Two-tailed Mann Whitney t-Test)



Anti-spore agglutinating antibody titer was significantly higher in Aspf3/Aspf9/LT1 vaccinated chickens given the vaccine MU, MU, MU versus non-vaccinated chickens (70 vs 10, P= 0.015), with no significant difference in antibody titer between birds vaccinated SC, MU,MU versus non-vaccinated birds (25 vs 10; P=0.127). (Two-tailed Mann Whitney t-Test)