“Attempts to Develop a Live Avian E. coli Vaccine”

Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is a multimillion dollar annual problem for the poultry industry due to mortality, lost production, and condemnations. Also important is the recent recognition that APEC may be a food-borne cause of human urinary tract infections. Therefore, control of avian colibacillosis may be important from both an animal health and a food safety standpoint. Because colibacillosis is a bacterial disease and has been thought to occur secondarily to concurrent infections and/or undesirable environmental factors, control of the disease has been previously attempted by use of antibiotics, farm sanitation and other management practices, eradication of *Mycoplasma*, and vaccination against respiratory viruses. However, *E. coli* respiratory tract infection/septicemia is still considered the most important bacterial disease affecting the poultry industry. In addition, the cost involved in the treatment, undesirable side effects, appearance of apparent transferable antibiotic resistance, and public concerns over the use of antibiotics in livestock limit the use of antibiotics. Therefore, control of the disease through vaccination is thought to be a logical and desirable approach. Other than the live attenuated vaccine from Fort Dodge Animal Health (which is still under investigation), there is no other vaccine currently available. Availability of an economical vaccine, effective against different strains of APEC, is essential to control colibacillosis.

The overall objective of this study was to reduce the poultry industry’s losses to colibacillosis through development of an effective vaccine which is capable of protecting chickens against both homologous and heterologous APEC challenge.

The specific objectives of this study were to:

- Create a live attenuated APEC vaccine by generating mutations in the selected genes of the pathogenicity island, PAI I<sub>APEC-O1</sub>, in the APEC O1 strain; (belongs to serotype O1:K1:H7);
- Assess the safety and immunogenicity of mutant strains by demonstrating that these mutants evoke strong antibody responses in the serum and air sac washings of inoculated birds and do not cause disease in these birds; and
- Assess the vaccine for its ability to protect chickens from an APEC challenge.
We created five different mutants of APEC O1 by deleting the entire pathogenicity island PAI $\text{APEC-O1}$ or specific regions located on the PAI $\text{APEC-O1}$.

We then assessed the level of attenuation, safety and immunogenicity of the mutant strains. To assess the attenuation of the mutants, one-day-old broiler chickens were inoculated with the vaccine strain via the subcutaneous route. To assess the safety of the mutant strains, two-week-old broiler chickens were exposed to the mutants via the respiratory route in aerosols. Only the $\text{pap}$ operon deleted strain and the PAI $\text{APEC-O1}$ deleted strain were attenuated and safe to use as vaccines. However, when the sera and air sac washings collected from two-week-old chickens vaccinated with these strains via the respiratory route were tested for their IgY antibody response, none of these mutants resulted in sufficient serum or mucosal antibodies (in air sac washings) to protect chickens against a challenge with APEC. Also, none of the vaccines we tested protected chickens from subsequent APEC challenge. Therefore, none of the mutants we made in this study are suitable as a vaccine to protect chickens from colibacillosis.

Although this study failed to develop a vaccine to protect chickens from colibacillosis as proposed, this work added to the current understanding of virulence mechanisms of APEC.

We confirmed that the $P$ fimbriae are involved in the pathogenicity of the APEC O1 strain and perhaps of the other APEC strains that carry the $\text{pap}$ operon.

We also determined that the $\Delta tia$ mutant strain failed to adhere to and invade a human ileo-cecal epithelial cell line as compared to the wild-type strain suggesting that $tia$ may be important for APEC persistence in the chicken gut.

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