Recombinant Orally Effective Vaccine Platforms Expressing Putative Conserved Antigens for Reduced Antimicrobial Usage in Poultry

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Salmonella: The Problem and Possible Solutions

• *Salmonella* is still a food borne problem in the US and EU
• Cases of *Salmonella* are commonly linked to poultry products
• Multiple intervention strategies have been tried
  • Therapeutic Antibiotics
  • Biosecurity
  • Probiotics and DFM
  • Vaccination (Killed or Live-Attenuated)
Vaccination

**Killed whole-cell bacterins**
- Systemic infections
- Can reduce organ invasion and shedding but limited in stopping mucosal colonization.
- Often requires multiple injections which is not always an option in poultry production.
- Primarily humoral response

**Live-attenuated vaccines**
- Can colonize mucosa (i.e. gastrointestinal tract)
- Can competitively exclude other bacteria (*Salmonella*)
- Can be delivered via ocular, nasal or oral administration routes
- Elicit secretory IgA, humoral and cell-mediated response
**Salmonella Vector**

- Double-attenuated SE PT13A or single-attenuated ST
- Plasmid inserted into the *lamB* gene (loop 9) of the genome to create a site for vaccine sequence insertion
- Many copies of lamB exist on cell surface

- Results in cell surface expression of the vaccine sequences
Bacillus Vector & Plasmid

- *Bacillus subtilis* 1A857
  - Contains *srtA* gene to anchor proteins on cell surface
- Plasmid pNDH10
  - Contains *fpbB* gene to make fibronectin binding protein, a sorting vector to allow binding of proteins to sortase A
Recombinant Vaccines – Cell Surface Expression

*Salmonella* Enteriditis- CJ0113-PAL-HMGB1
Rabbit polyclonal HMGB1 (90-111) + HMGB1 (156-177)
Antigenic Sequence Selection

- Highly conserved
- Antigenic & immunogenic
- B-cell & T-cell stimulatory
- Immunoprotective
High-mobility group box 1 (HMGB1)

- Passively released from necrotic cells
  - HMGB1 release
  - TNF-α release
- Actively secreted by monocytes, macrophages, and natural killer cells
  - Secreted after IL-1β and TNF-α
- Outside of the cell it binds with high affinity to the receptor for advanced glycation end products (RAGE) response
- Potent mediator of inflammation

Ulloa and Messmer 2006
Salmonella: Antigenic Sequences

- PAL - peptidoglycan-associated lipoprotein
  - Highly conserved in Gram-negative bacteria
  - Expressed on outer membrane
- Cj0113 – Campylobacter
  - Omp18
  - Outer membrane protein homologous to PAL
Salmonella Constructs

- Steric hindrance optimal protein folding
  - *Salmonella*-CJ0113-PAL-HMGB1 (CPH)
  - *Salmonella*-HMGB1-CJ0113-PAL (HCP)
  - *Salmonella*-CJ0113-HMGB1-PAL (CHP)

Unfolded

Folded
Salmonella Vaccine Constructs

- Cj0113 + PAL + HMGB1
- Cj0113 + HMGB1 + PAL
- HMGB1+ Cj0113 + PAL

Cj0113  HMGB1
PAL  SPACER
Objectives

• Test all 6 construct configurations against a S. Heidelberg challenge
• Determine if one or more of the vaccine candidates could provide cross-serogroup protection
• Determine if these vaccine candidates could be used as an orally effective live-attenuated vaccine against *Salmonella*
Experiment 1

**Treatment Groups (n=20)**

- Negative Control
- SE-CPH
- SE-CHP
- SE-HCP
- ST-CPH
- ST-CHP
- ST-HCP

**Salmonella Heidelberg challenge**

- 6x10^6 cfu/chick

**Day 0**

- Primary Vaccination
  - 1x10^6 cfu/chick

- Boost Vaccination
  - 1x10^6 cfu/chick

**Day 23**

- Collect Cecal Samples

**Day 28**
Recovery of *Salmonella* Heidelberg from ceca after vaccination by oral gavage on day of hatch and 14 days of age

*Significantly different (P<0.05) than Control*
Experiment 2
**Treatment Groups (n=20)**

Negative Control
SE-CPH
SE-CHP
SE-HCP

*Salmonella* Heidelberg challenge
7x10^6 cfu/chick

Primary Vaccination
1x10^8 cfu/chick

Day

0  7  28  35

Collect Cecal Samples
Recovery of *Salmonella* Heidelberg from ceca after vaccination by oral gavage on day of hatch

*Significantly different (P<0.05) than Control*
Experiment 3
Treatment Groups (n=50)
Negative Control
SE-CPH

Salmonella Heidelberg challenge
3x10^7 cfu/chick

Primary Vaccination
1.6x10^7 cfu/chick
Spray vaccination

Collect Cecal Samples

Day
0
14
18
21
25
Recovery of *Salmonella* Heidelberg from ceca after vaccination by spray on day of hatch

*Significantly different (P<0.05) than Control*
Bacillus-vectored *Eimeria* Constructs

- **TRAP + HMGB1 (252aa)**
  - TRAP: AAPETRAVQPKPEEGHERPEPEEEEEEKKEEGGGFPTAAVA
  - HMGB1
  - Spacer

- **MPP + HMGB1 (255aa)**
  - MPP: PSHDAPESERTPRVISFGYGACEHNLGVSLFRREETKKDPRGR
  - HMGB1
  - Spacer

- **TRAP + MPP+HMGB1 (302aa)**
  - TRAP: AAPETRAVQPKPEEGHERPEPEEEEEEKKEEGGGFPTAAVA
  - MPP: PSHDAPESERTPRVISFGYGACEHNLGVSLFRREETKKDPRGR
  - HMGB1
  - Spacer
**Eimeria** challenge

**Total mortality post-Eimeria challenge**

*P<0.05  **modified chitosan adjuvant*
Discussion & Conclusions

• SE-CPH vaccine was able to provide cross-serogroup protection against a heterologous SH challenge
• ST-HCP vaccine was able to provide cross-serotype protection from the SH challenge and homologous serogroup protection
• *Bacillus* vectored vaccine was able to relay protection against *E. maxima* challenge in broilers
• Persistence of protection requires further work
• Vectors need optimization to make them ideal for commercial use
  • Antibiotic resistance in plasmid (*Bacillus*)
  • Clearance and monitoring (*Salmonella*)
THANK YOU