Strategies for Controlling the Risk of Foodborne Pathogens and Antibiotic Resistance

J. Allen Byrd PhD, DVM
Director, Poultry Food Safety
Real World Challenges

• Antibiotics
• High levels of *Salmonella* in hatchery
• Heavily contaminated litter
• Biosecurity issues (rodents, wild birds, etc)
• Feed deprivation
• Dirty live-haul cages
• Processing plant
Biosecurity

Location: Isolate farms from other poultry and livestock. Single-age sites are preferable to limit recycling of pathogens and live vaccine strains.

• Farm design: A barrier (fence) is necessary to prevent unauthorized access. Design housing to minimize traffic flow and to facilitate cleaning and disinfection. Construct housing to be bird and rodent proof.

• Operational procedures: Prevent the introduction and spread of disease with procedures that control the movement of people, feed, equipment and animals on the farm. Routine procedures may have to be modified in the event of a change in disease status.
Pre-harvest Intervention strategies

- **Probiotic Strategies**
  - Competitive exclusion
  - Probiotics
  - Prebiotics

- **Antibiotic Strategies**
  - Antibiotics
  - Bacteriophage
  - Specific inhibition of pathways
  - Immunization

- **Diet and management**
  - Dietary switch
  - Water troughs
  - Other management factors
Factors that disturb the barrier

• Any stress that causes chickens not to eat
  – environment
  – delayed access to feed or water
  – feed or water deprivation
  – change in feed composition
  – vaccination
  – disease

• Antibiotics
Distinction between competitive exclusion cultures and probiotics

- **Competitive exclusion cultures**
  - Consist of beneficial bacteria native to the host animal
  - Considered drugs by FDA and subject to regulation

- **Probiotics (Direct-Fed Microbials)**
  - Consist of GRAS organisms, typically Bifidobacteria, Lactobacilli, Yeast cultures
How does CE work?

- Direct competition for attachment sites
- Direct competition for nutrients
- Production of inhibitory products or conditions (e.g., volatile fatty acids, lower pH, changed $E_h$, bacteriocins or colicins)
- Stimulation of immune system
- Combinations of some/any of the above.
Cecal crypts (Arrowheads) appear unobstructed by colonized bacteria in both mid (A) (#6314) and distal (B) (#6317) ceca compared to similar sections from PREEMPT™ treated chicks (Fig. 1). Strands of mucous (Arrows) interconnect adjacent crypts (Arrowheads) in the distal ceca without the associated bacteria noted in Fig. 1B. Bars = 100µm.
Colonized bacteria are evident within cecal crypts (Arrows) in both the mid (A) (#6284) and distal (B) (#6282) cecum. Within the distal cecum large numbers of bacteria are evident entangled within a matrix of mucous which interconnects adjacent crypts (Arrowheads). Bars = 100µm.
Feed Withdrawal

• Evacuate the crop and reduce pressure on GI tract in order to minimize rupture (Summers & Leeson, 1979).

• Minimize the loss of feed not absorbed by the digestive tract and converted to muscle (Brown, 1963).
Changes in Crop

• During an 8 hour feed withdrawal:
  – Crop pH increases
  – *Lactobacilli* numbers decrease
  – Lactic acid concentrations decrease (Corrier *et al.*, 1999b).
  – Consumption of possible pathogen-contaminated litter/feces.
## Feed withdrawal & crop Salmonella

<table>
<thead>
<tr>
<th>Length of FW</th>
<th>Salmonella (+) drag swab</th>
<th>Pre-FW Salmonella</th>
<th>Post-FW Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0/4</td>
<td>0/40</td>
<td>0/40</td>
</tr>
<tr>
<td>4</td>
<td>0/4</td>
<td>0/40</td>
<td>0/40</td>
</tr>
<tr>
<td>5</td>
<td>0/4</td>
<td>0/40</td>
<td>0/40</td>
</tr>
<tr>
<td>7</td>
<td>4/4</td>
<td>3/40 (7.5)</td>
<td>14/40 (35)</td>
</tr>
<tr>
<td>8</td>
<td>3/4</td>
<td>0/40</td>
<td>3/40 (8)</td>
</tr>
<tr>
<td>8</td>
<td>3/4</td>
<td>0/40</td>
<td>4/40 (10)</td>
</tr>
<tr>
<td>8</td>
<td>0/4</td>
<td>0/40</td>
<td>2/40 (5)</td>
</tr>
<tr>
<td>8</td>
<td>0/4</td>
<td>0/40</td>
<td>1/39 (2.6)</td>
</tr>
<tr>
<td>8</td>
<td>4/4</td>
<td>4/40 (10)</td>
<td>12/40 (30)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7/360 (1.9%)</strong></td>
<td><strong>36/359 (10%)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Effect of feed removal on the tendency of broilers in five commercial flocks to feed on litter

<table>
<thead>
<tr>
<th>Flock</th>
<th>Pre-FW</th>
<th>Post-FW</th>
<th>Increase (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>112</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>199</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>322</td>
<td>4.2</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>286</td>
<td>10.6</td>
</tr>
<tr>
<td>5</td>
<td>115</td>
<td>333</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Average Counts</strong></td>
<td><strong>61</strong></td>
<td><strong>250</strong></td>
<td><strong>4.1</strong></td>
</tr>
</tbody>
</table>
Fluorescein Dye
Fluorescein Dye
Pest Management

• Darkling beetles were only positive in the presence of positive flocks. And no positive beetles were detected between flocks. (Skov et al., 2004)

• Determined that genetically distinct isolates were found to be common with the beetles and the broilers suggesting that beetles may be a reservoir for *Salmonella*. (Bates et al, 2004)
Lower Stress Response

• Less Stress Impact
  – Stronger Protection
  – Reduced Inflammation
  – Milder Corticosterone Release
  – Faster Recovery
Stress on the Host and the Pathogens

- Virulence
- Mucosal Attachment

Pathogen

Host

Horizontal Transmission

Fecal Shedding

Inflammation and translocation

Environment

Fecal Shedding

Replication
Stress Perceived by the Animal

**“Fight or Flight”**
- Epinephrine or Norepinephrine
- Short term
- Self preservation
- Abandonment of reproduction

**“Long term Stress”**
- Corticosterone
- Decrease BW
- Increase fat deposition
- Decrease muscle tissue
- GI changes
- Egg production reductions
Catabolism Caused by Corticosterone (Stress)

- Stress
- Increased Fatty Acids
- Decreased Body Weight
- Loss of body mass
- Liver
  - Protein Synthesis
  - Gluconeogenesis
  - Uric acid
  - Fuel for brain, RBC and kidneys
- Muscle Breakdown
  - Amino Acids
- Glycolysis utilization
  - Increased Glucose
  - Increased calcium and sodium loss
  - Increased osteoporosis
- Increased leg problems
- Ketones for brain
- Density
- Nutritional
- Weather
- Human
- Predators
- Illness
Gastrointestinal Changes Due to Stress

- Faster passage time
- Lower Protein absorption
- Lower Carbohydrate absorption
- Changes in the Protective Microbiota
- Increased Intestinal permeability
- Increased Calcium loss
- Other nutrients lost

Feed intake (16.4%)
Feed conversion (25.6%)
Body weight (32.6%)

Egg parameters
- Thickness
- Weight
- Breakage

Molnar and Gair, 2012
Virus Protection

• Improved Health Benefits
  – Protection from Virus Challenge
  – Lower Virus Shedding
  – Better Vaccine Protection
  – Reduced Clinical Signs
Effects of Stress on the Immune Cells

Median LPAI Titer Levels: Overall Challenge

1. LPAI Challenge at 28 days of age

Linke et al., 2018
Pathogen Mitigation

• Pathogen Reduction Inside the Bird
  – Lower Prevalence ( # Positive Birds)
  – Reduced Pathogen Numbers
  – Less Pathogen Shedding
  – *Salmonella, E. coli, and Campylobacter*
Portfolio of Foodborne Pathogen Research

- **Salmonella**
  - Arizonae
  - Enteritidis
  - Gallinarum
  - Heidelberg
  - Infantis
  - Java
  - Kentucky
  - Newport
  - Pullorum
  - Typhimurium

- **Campylobacter**
  - coli
  -jejuni

- **E. coli**
  - APEC: Avian Pathogenic
  - STEC: Shiga Toxin-producing

Proven Research:
- Pathogen Challenge:
  - In vitro
  - Pens
  - Field
- Environmental Samples
- Processing Plant
- Further Processing
  - Parts
  - Ground
- Virulence
- Antibiotic Resistance
Market Age Broiler Ceca Content Load

- Each bird contains two cecum that each have 7 mL/gm of cecal contents.
- So, 14 mLs/gm per bird that can be evacuated 2-3 times per day.
- Therefore, each bird contributes 42 mLs of cecal contents per day to the broiler house!

- A 20,000 bird house will produce 840,000 mLs/gms of cecal contents.
- If 20% of birds were *Salmonella* positive, then 168 kg or 370 lbs of *Salmonella* positive ceca contents are dispersed.

168 Kg/house/day of *Salmonella* positive contents
Salmonella in a Broiler House

• If 168,000 g of ceca contents are dispersed in a broiler house per day and each gram contains 1 million organisms
  – 168 billion (11.23 Log$_{10}$ Salmonella CFU) are spread every day

• By reducing 1.6 Log$_{10}$ Salmonella CFU/day

• The number of Salmonella will be reduced to 4.3 billion organisms per day.
**Salmonella Prevalence in Rehang Carcass Rinses (46d): Broilers**

<table>
<thead>
<tr>
<th>Period</th>
<th>Treatment</th>
<th>#Positive / # Tested</th>
<th>Prevalence (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>July-August</td>
<td>CON</td>
<td>17/200</td>
<td>8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>XPC</td>
<td>1/200</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

- Original XPC Inclusion Rate: 1.25 kg/tonne
- Carcass rinsate from 25 birds per barn
  - (200 birds per treatment)
- 8 CON and 8 XPC houses
Dot Plot of *Salmonella* MPN from Culture-Positive Rehang Carcass Rinses by Treatment

July-Aug 2014

Load is actually the **SUM** of samples

Control  Treatment  XPC

N = 17  N = 1

Broilers; Field Trial/UGA-PDRC, 2014
In Commercial Field Evaluations

**Salmonella Prevalence (%) vs. Control**

Avg. = 54.1 % Reduction

N = 12,046 samples

Field Trials: AL, AR, CA, GA, IA, IN, ME, MN, MO, NC, OH, SC, SD, TX, VA
In Commercial Field Evaluations

**Salmonella Numbers** (CFU/g) vs. Control

- Broilers
- Turkeys
- Layers

Field Trials: AL, AR, CA, GA, IA, IN, ME, MN, MO, NC, OH, SC, SD, TX, VA

N = 12,046 samples

Avg. = 86.8% Reduction
• Restored Pathogen Sensitivity to Antibiotics
  – Pathogens Previously Resistant
  – Genetic Mechanism Expelled
  – Pathogens Sensitive to Antibiotics
  – Antibiotic Treatments More Effective
Cobb 500 broilers were infected with *Salmonella* Typhimurium DT104 on Days 2, 9, and 16.
- DT104 is resistant to 5 types of antibiotics.
- *Salmonella* shedding was quantitated on Days 28, 35, and 42.
- *Salmonella* intestinal colonization was quantitated on Day 49.
- Antibiotic resistance of the recovered *Salmonella* was assessed on Days 28, 35, 42, and 49.
- Study repeated 3 times and published in Poultry Science.
Antibiotic Resistance of *Salmonella* Typhimurium

Feeding XPC reduced antibiotic resistance

75% Antibiotic Resistant

CON\textsuperscript{a}

15% Antibiotic Resistant

XPC\textsuperscript{b}

\(a, b P < 0.05\)

\(\bullet = \text{Salmonella} \quad \bullet = \text{Antibiotic-Resistant Salmonella}\)

Feye et al., 2016
Poultry Science 95: 2902-2910
Percent Positive *Salmonella* by Sample Type

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Percent Positive</th>
<th>Sample Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>47%</td>
<td>2121</td>
</tr>
<tr>
<td>Traypad</td>
<td>21%</td>
<td>303</td>
</tr>
<tr>
<td>Drag Swab</td>
<td>13%</td>
<td>304</td>
</tr>
<tr>
<td>Litter</td>
<td>10%</td>
<td>2099</td>
</tr>
<tr>
<td>Ceca</td>
<td>4%</td>
<td>2099</td>
</tr>
<tr>
<td>Crop</td>
<td>23%</td>
<td>2099</td>
</tr>
<tr>
<td>Whole Carcass</td>
<td>40%</td>
<td>272</td>
</tr>
<tr>
<td>Drag Swab</td>
<td>35%</td>
<td>272</td>
</tr>
<tr>
<td>Litter</td>
<td>16%</td>
<td>1979</td>
</tr>
<tr>
<td>Ceca</td>
<td>18%</td>
<td>1978</td>
</tr>
<tr>
<td>Crop</td>
<td>51%</td>
<td>1979</td>
</tr>
<tr>
<td>Whole Carcass</td>
<td>44%</td>
<td>1974</td>
</tr>
<tr>
<td>PreChill</td>
<td>24%</td>
<td>1920</td>
</tr>
<tr>
<td>PostChill</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pre-Chill *Salmonella* Status at the Flock Level

The best predictors for Pre-Chill (45%) are:

**Plant Arrival**
- Whole Carcass Rinse and Crop

**Post-Harvest**
- Litter and Drag Swabs

**Day 1**
- Litter
A Final Word

The complete intervention program begins prior to hatch and ends with proper cooking and handling of the product by the consumer.
XXV CONGRESO CENTROAMERICANO Y DEL CARIBE DE AVICULTURA
HONDURAS 2018

21, 22 y 23 DE AGOSTO, 2018
CENTRO DE CONVENCIONES COPANTL, SAN PEDRO SULA