Salmonella in Eggs: Issues and Research

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S. enteritidis Outbreaks in the USA

Number of outbreaks

Year

85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 00 01 02

Number of outbreaks
The association of eggs with human *Salmonella enteritidis* and *S. heidelberg* infections:

- 80% of human *S. enteritidis* outbreaks in the USA (1985-1999) for which a food source could be identified were attributed to eggs or egg-containing foods.

- 23% of *S. heidelberg* outbreaks in the USA (1973-2001) were attributed to eggs or egg-containing foods.
The deposition of *Salmonella* in eggs is a consequence of the colonization of reproductive organs, particularly the ovary and upper oviduct, in systemically infected hens.
Salmonella Recovery from Reproductive Tissues and Egg Contents

% positive

Salmonella Recovery from Reproductive Tissues and Egg Contents

<table>
<thead>
<tr>
<th></th>
<th>Ovaries</th>
<th>Oviducts</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH 1</td>
<td>30</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>SH 4</td>
<td>20</td>
<td>25</td>
<td>5</td>
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<tr>
<td>SH 5</td>
<td>15</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>SH 11</td>
<td>5</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>SE 6</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tbody>
</table>
What is the incidence of *S. enteritidis* infection in laying flocks?

7% of commercial laying houses in the USA were environmentally positive for *S. enteritidis* in a 2000 survey conducted by the USDA National Animal Health Monitoring System.
What is the incidence of *S. enteritidis* contamination of eggs?

In field studies of environmentally positive flocks in Pennsylvania and California, the prevalence of egg contamination with *S. enteritidis* was approximately 5 in 20,000 (0.025%).

The 1998 USDA *S. enteritidis* Risk Assessment Report estimated the overall national egg contamination prevalence to be 1 in 20,000 (0.005%).
Recent Events in S. enteritidis
Epidemiology and Control

- January, 2005: The Risk Assessment Division of USDA-FSIS estimates that 182,060 human illnesses were caused by consumption of S. enteritidis-contaminated shell eggs in 2000.

- September, 2004: FDA proposes regulations for shell egg production that include mandatory testing of environmental samples (and eggs, if necessary) from commercial laying flocks.
Common Risk Reduction Practices for S. enteritidis in Poultry Flocks

- Using uninfected chicks
- Effective pest control
- Cleaning and disinfection between flocks
- Heightened biosecurity
- Washing and refrigeration of eggs
- Vaccination
Changes in Human S. enteritidis Incidence in 31 States after Implementation of Egg Quality Assurance Programs

% of states

Decreased
Increased

Source: CDC
Proposed FDA Regulations for Shell Egg Producers

- Purchase chicks from uninfected (NPIP-certified) breeder flocks.
- Maintain pest control and biosecurity programs.
- Test laying house environmental samples for *S. enteritidis* at 40-45 weeks of age and after induced molting.
- If environment positive for *S. enteritidis*, clean/disinfect thoroughly between flocks and test eggs. If eggs positive, divert for pasteurization.
- Store eggs under refrigeration at 45° F.
Recovery of S. enteritidis from eggs laid by experimentally infected hens

Weeks post-inoculation

% positive

Weeks post-inoculation

Shells
Yolks
Albumens
What are the research needs of regulatory agencies and the poultry industry regarding *Salmonella* in eggs?

Sources of Information:

- 2004 USDA-FSIS Risk Assessment for *Salmonella enteritidis* in Eggs
- Egg Nutrition Center Food Safety Advisory Panel
- American Egg Board Open House
Post-Harvest (Egg Processing) Research Needs

- Prevalence of pathogens on egg shells before and after processing
- Effectiveness of egg sanitizers
- Validation of current and prospective regulatory provisions
Egg Processing Safety, Quality, and Security

Deana Jones – Food Technologist (Lead Scientist)

Michael Musgrove – Food Technologist
Effects of commercial cool water washing on pathogen detection in shell eggs

- Egg surface temperature was significantly reduced when eggs were washed with a combination of warm and cool water.
- *Enterobacteriaceae* frequency was not different between wash water temperature treatments for offline eggs.
- *Listeria* was detected in cool wash water at the offline facility but not on or in any eggs.
- *Salmonella* and *Campylobacter* were detected within the shells of 3 and 2 (of 384) samples, respectively. All positive samples were from cool water treated eggs.
- More work needs to be conducted to determine the feasibility of cool water washing of shell eggs, including examining detergents designed for cooler temperatures.
Correlation of shell strength and *Salmonella* Enteritidis infection

- Eggshell quality has always been difficult to quantify due to variable nature of eggs
- Advent of more objective detection devices has eliminated much of the testing variability
- A highly invasive inoculation method was utilized, yet it was very difficult to detect SE in the egg. Positive egg contents were only found after pre-enrichment.
- Correlation analysis did not find any strong positive or negative correlations between shell strength and SE contamination within the range of shell strengths detected in the current study
Recovery of *Salmonella* from commercial shell eggs by two methods

- Utilized shell rinse and shell crush and rub
- Monitored for naturally occurring *Salmonella*
- Shell rinse recovered 4.8% positives
- Shell crush and rub recovered 5.3% positives
- Occasionally only one method was positive for *Salmonella* per sample
- Using both methods increased sensitivity, although in most cases, crushing provided more sensitive detection
Impact of commercial processing on the incidence of *Salmonella* on shell eggs

<table>
<thead>
<tr>
<th><em>Salmonella</em></th>
<th>No. of isolates</th>
<th>Sample type</th>
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</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>21</td>
<td>Eggshell</td>
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<tr>
<td></td>
<td>1</td>
<td>Tap water</td>
</tr>
<tr>
<td>Typhimurium (Copenhagen)</td>
<td>4</td>
<td>Eggshell</td>
</tr>
<tr>
<td>4-12:i:-monophasic</td>
<td>2</td>
<td>Eggshell</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>9</td>
<td>Eggshell</td>
</tr>
<tr>
<td>Kentucky</td>
<td>1</td>
<td>Eggshell</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Wash water</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39</strong></td>
<td></td>
</tr>
</tbody>
</table>
Pre-Harvest (Egg Production) Research Needs: Stress and Immunity Issues

- Effects of induced molting (by feed withdrawal and alternative methods) on immune responsiveness and susceptibility to *Salmonella* infection
- Stress associated with other management practices and effects on immune responsiveness
- Protection by vaccination and stress effects on vaccine efficacy
Stress Effects on Immunity and Physiology of Poultry

Peter Holt – Immunologist
(Lead Scientist)

Randle Moore – Veterinarian/Physiologist
Effect of Feed Deprivation and Recovery from Feed Deprivation Over Time on:

Intestinal Length

Intestinal Weight

- Full Fed
- Fasted
- Full Fed Dry
- Fasted Dry
Effect of Feed Deprivation on *in Vitro* Tissue Invasion of *Salmonella enteritidis*

**Ovary**

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
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</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
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**Crop**

<table>
<thead>
<tr>
<th>Trial 1</th>
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<tbody>
<tr>
<td>a</td>
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<td>a</td>
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<tr>
<td>a</td>
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</table>

**Ileum**

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<td>a</td>
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</table>

Legend:
- **Full Fed**
- **Non-Fed**
Effect of Induced Molting on Levels of SE in Flies vs on Floor

SE levels in flies

SE levels on floor
## Isolation of *S. Enteritidis* from Fly Interior vs Exterior

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exterior</th>
<th>Interior</th>
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<tbody>
<tr>
<td>Nonmolt</td>
<td>8/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Molt</td>
<td>9/10</td>
<td>9/10</td>
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</tbody>
</table>

## Isolation of *S. Enteritidis* from Internal Organs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sal. Gland</th>
<th>Crop</th>
<th>Gut</th>
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</thead>
<tbody>
<tr>
<td>Nonmolt</td>
<td>0/10</td>
<td>1/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Molt</td>
<td>0/10</td>
<td>3/10</td>
<td>8/10</td>
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Pre-Harvest (Egg Production) Research Needs: Pathogenesis Issues

- Prevalence, level, and site of egg contamination; growth kinetics of *Salmonella* in eggs during storage; effects of refrigeration on bacterial growth in eggs
- The evolution (and identification) of *Salmonella* strains able to contaminate eggs
- Genetic differences between *Salmonella* strains that can be used to establish epidemiological relationships (including sources and routes of transmission)
Controlling Egg Contamination with Salmonella enterica by Understanding its Evolution and Pathobiology

Jean Guard-Bouldin – Veterinarian/Molecular Biologist (Lead Scientist)

Richard Gast - Microbiologist (Research Leader)
Mean Duration of Salmonella Recovery from Fecal Samples

days postinoculation

Salmonella Recovery from Eggs

% positive

Parent strains
Passaged strains

Parent strains
Passaged strains
S. enteritidis penetration into egg yolks

% positive

- PT8
- PT13a

- 7° / 24 hours
- 30° / 24 hours
Phenotype can vary dramatically within serotype and phage type

PT13A

Egg Contamination

biofilm negative

PT13A

≠ Egg Contamination

biofilm positive
Work in progress from Jean Guard Bouldin, DVM, Ph.D., USDA-ARS-ESQRU, Athens GA
Detecting and protecting against specific Salmonella serotypes can be essential for responding to urgent or severe threats to public health, but the most cost-effective and sustainable approaches to controlling food-borne disease are based on risk reduction practices that address a broad spectrum of current and emerging pathogens.