Strategies for Controlling Salmonella Enteritidis in Poultry Flocks: Translating Research into Action

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“An expert is a person who has made all the mistakes that can be made in a very narrow field.”

---Niels Bohr---
“It has long been recognized that salmonellae occur throughout the world, that they commonly infect food producing animals, with or without development of serious disease, and that raw foods of animal origin are frequently contaminated by salmonellae and serve as a major source of human infection. A variety of approaches are being used in different parts of the world to prevent or contain infections in domestic food animals. Marked broad success has been achieved only with the host-adapted species *Salmonella pullorum* and *S. gallinarum* in poultry.”

---G. H. Snoeyenbos---

*(Preface to Proceedings of AAAP International Symposium on Salmonella, 1984)*
**Challenges to Controlling Salmonella in Poultry**

- *Salmonella* infections of poultry can be inapparent.
- Newly hatched poultry are highly susceptible to *Salmonella* colonization.
- Many *Salmonella* serotypes have a very wide host range and can be environmentally persistent.
- Poultry houses often contain materials which are difficult to clean and disinfect. Manure and dust are present in large quantities.
Numerous *Salmonella* serotypes have been identified, but only a small proportion of these have ever been isolated from poultry (and even fewer are common).

A small number of serotypes accounts for most human salmonellosis, and many of the predominant human serotypes are similarly prevalent in poultry.
Preventing Human Infections with Food-borne Salmonella

Postharvest:
Safeguard the microbial integrity of food products or decontaminate them before consumption.

Preharvest:
Minimize opportunities for the introduction, persistence, and transmission of flock infections with *Salmonella*. 
Preharvest Control Strategies for Salmonella in Poultry Flocks

- Serotype-Independent: Broadly directed against all serotypes (and in some instances against other microorganisms as well).
- Serotype-Specific: Targeted to act with precision against particular serotypes with distinctive public health or economic significance.
The Association of Eggs with Human Salmonella Infections in the USA

The Centers for Disease Control attributed 81% of human *S. Enteritidis* outbreaks (1985-2002) and 23% of *S. Heidelberg* outbreaks (1973-2001) which had identifiable sources to eggs or egg-containing foods.

A U.S. Department of Agriculture risk assessment report estimated that >100,000 human illnesses may be caused annually by the consumption of shell eggs contaminated by *S. Enteritidis*. 
2010 S. Enteritidis Outbreak in the USA

- From May 1 to October 15, ~1,813 outbreak-associated illnesses were reported (with a single PFGE pattern).
- 29 “event clusters” (>1 illness) were identified in 11 states. A single Iowa producer supplied eggs to 15 of these clusters. Another producer was also implicated.
- ~600 environmental samples were tested (manure, walkways, equipment, surfaces, feed): 11 samples were positive for the outbreak strain (+1 wash water isolate from an in-line egg processing facility).
- ~550 million eggs were recalled.
**Objective:** Review four prominent categories of options for preharvest control of Salmonella in poultry flocks to evaluate the opportunities and limitations generated by application of these strategies (individually or in combination).

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Testing</th>
<th>Colonization Control</th>
<th>Management &amp; Sanitation</th>
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</thead>
</table>

Most serotype-specific  Least serotype-specific
Part I: Vaccination

Student Art work from Chicken Art Project, Dubuque (IA) Community Schools
Goals of Salmonella Vaccination in Poultry Flocks

- Reduce the susceptibility of individual birds to infection.
- Reduce horizontal transmission of infection within flocks.
- Reduce vertical transmission of infection to progeny of breeding flocks.
- Reduce the *Salmonella* load in poultry house environments (and the likelihood of transmission to subsequent flocks).
- Reduce the frequency of product contamination.
Inactivated (Killed) and Attenuated (Live) Salmonella Vaccines

- An advantage of inactivated vaccines is that autogenous bacterins can be rapidly prepared from specific strains responsible for problems in a particular locale or in a particular commercial enterprise or industry.

- Advantages of attenuated vaccines include their ease of administration and their greater ability to induce a strong and long-lasting cellular immune response.

- Both the opsonic activity of antibodies and the phagocytic and lytic activities of cellular effectors may be required for the full expression of immunity.
Efficacy of Salmonella Vaccines

- Significantly fewer human S. Enteritidis infections were reported in the U.K. after the widespread implementation of a vaccination program for egg-laying hens.

- Vaccinating U.K. laying flocks lowered the frequency of egg contamination with S. Enteritidis even when laying-house environments remained contaminated.

- Both killed and live vaccines have provided significant protection against *Salmonella* infection, but neither type of vaccine has consistently been able to prevent infection entirely (especially against high challenge doses) or effectively cross-protect against heterologous serotypes.
Protection of Laying Hens against S. Enteritidis by a Killed Vaccine
Efficacy of Salmonella Vaccines (continued)

- Poor vaccine performance has been associated with severe rodent control or sanitation problems in poultry houses, feed or water deprivation, and environmental stresses such as heat.

- The protective efficacy of vaccination is less dependable against serotypes that are antigenically dissimilar to the strains used for vaccine preparation, so vaccination is unlikely to prevent the emergence of new problems associated with previously inconsequential serotypes.
Vaccination may be most valuable as a component within a comprehensive program of risk reduction practices, especially in application to highly susceptible flocks or flocks exposed to severe challenges from environmental sources, when epidemiologic evidence has led to heightened concerns about particular *Salmonella* serotypes.
Part II: Testing
An Example of a Successful Testing Strategy:

Pullorum Disease Testing Summary for Chickens in Commercial Flocks in the USA

<table>
<thead>
<tr>
<th>Year</th>
<th>% Positive Tests</th>
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<tbody>
<tr>
<td>1935-36</td>
<td>3.66</td>
</tr>
<tr>
<td>1986-87</td>
<td>0.000005</td>
</tr>
</tbody>
</table>

(Source: National Poultry Improvement Plan, 1988)
An Example of an Unsuccessful Testing Strategy:

A trace-back testing program for *S. Enteritidis* was applied to laying flocks in the U.S. from 1990-1995: restrictions were imposed on 31 flocks, 9 million laying hens were depopulated, and >1 billion eggs were diverted for pasteurization.

**Recovery of *S. Enteritidis* in USDA Surveys**

<table>
<thead>
<tr>
<th>Year</th>
<th>Spent Hens</th>
<th>Liquid Eggs</th>
</tr>
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<tbody>
<tr>
<td>1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% positive

100 90 80 70 60 50 40 30 20 10 0

1991 1995
Trace-back testing is unlikely to achieve the identification and eradication of all infected flocks when *Salmonella* can be continuously reintroduced from diverse environmental sources.

Decision-making based on testing results always involves some uncertainty because of both test sensitivity issues and temporal variations in detectable parameters of infection.
Egg Contamination, Fecal Shedding, and Egg Yolk Antibodies (Hens Infected with S. Enteritidis)
In *Salmonella* control programs with testing and response components, the effectiveness of the response depends on the nature of the testing questions that are asked:

- What samples are collected and how are they tested?

Testing in quality assurance programs serves both to detect flocks that pose a potential threat to public health and to verify that the investment of resources in risk reduction practices is cost-effective.
Basing decision-making and response on serotype-specific testing results is useful, arguably even essential, for responding to severe public health problems that are tightly linked to individual serotypes, but a more serotype-independent approach has the advantage of detecting and responding to emerging problems (perhaps involving new reservoirs of infection or previously uncommon serotypes) before their impact becomes more severe.
Part III: Gastrointestinal Colonization Control
Fecal Shedding of S. Enteritidis by Laying Hens Inoculated Orally at 1 Day of Age

% positive

Age (in weeks)

Trial A

Trial B
The complete normal intestinal microflora of mature poultry protects against *Salmonella* colonization by both direct steric interference with *Salmonella* attachment to the intestinal epithelium and indirect inhibition of *Salmonella* growth in the gut.

Providing young chicks with intestinal contents from mature birds or undefined anaerobic cultures derived from this material has reduced both intestinal colonization and invasion to internal tissues by *Salmonella*.

Defined mixtures of microorganisms offer potentially greater consistency of performance and assurances of safety, but typically offer less protective efficacy than undefined preparations.
Colonization control is most effective when chicks or poults are treated before exposure to pathogens, so the presence of salmonellae in hatcheries or rearing facilities can severely compromise the value of competitive exclusion treatments.

The greatest opportunity for protection by competitive exclusion treatment is during the first few days of life when susceptibility to infection is highest and *Salmonella* exposure can lead to very persistent infection.

Prophylactic provision of a mature intestinal microflora to mature birds is of uncertain value except when the normal microflora are compromised.
Chemical additives in feed or water can be used to reduce bacterial counts and prevent re-contamination as well as to manipulate intestinal biochemistry to either directly inhibit pathogen colonization or support the growth of protective microflora.

- Acids (formic, propionic, lactic, butyric, caproic)
- Complex carbohydrates (lactose, mannose, glucose, sucrose, fructooligosaccharide)
- Chlorate
Like vaccination, colonization control is seldom able to completely exclude *Salmonella* and its effects can be overcome by severe *Salmonella* challenges. Nevertheless, colonization control can make a significant contribution to risk reduction efforts by diminishing susceptibility to *Salmonella* infection in vulnerable young poultry and during periods of stress for flocks when the normal intestinal microflora can be disrupted.
Part IV: Flock Management and Sanitation
The proportion of environmental samples positive for *S. Enteritidis* correlated with the prevalence of egg contamination.

Heavy mouse infestation was associated with a significantly higher likelihood of environmental contamination with *S. Enteritidis*.

Only 50% of houses with positive environments were converted to negative status by cleaning and disinfection.
Management and Environmental Risk Factors for S. Enteritidis Infection in Poultry Flocks

- High housing density
- Large flock size
- Windowless houses
- Multiple age complexes
- Outdoor runs
- Deep litter manure handling systems
- Automatic feeders
- Watering system
- Low ventilation rates
- Vertical cage stacks
- Floor rearing
- Cage rearing
- Flock age
- Induced molting by feed restriction
Management and Sanitation Issues for Salmonella in Poultry Flocks

- Hatcheries - where young birds are at their stage of maximum susceptibility to infection - are especially critical Salmonella control points.

- *Salmonella* can survive in litter and feed for more than 2 years after the removal of an infected flock.

- Levels of moisture in poultry houses are highly influential in determining whether salmonellae can persist.
Salmonella isolated from finished carcasses or table eggs have been occasionally -- but not frequently -- linked to contaminated feeds.

Rodents (mice and rats) and insects (flies, litter beetles, and cockroaches) are constant threats to perpetuate and amplify environmental contamination and to re-introduce Salmonella after cleaning and disinfection of poultry houses.

Feed withdrawal during induced molting or transportation can significantly increase the susceptibility of poultry to Salmonella infection.
Common Risk Reduction Practices in Salmonella Control Programs

- Eggs and chicks (or poults) should be obtained only from breeding flocks proven to be Salmonella-free.
- Hatching eggs should be properly disinfected and hatched under conditions of stringent sanitation.
- Poultry houses should be thoroughly cleaned and disinfected between flocks using recommended procedures.
- Rodent and insect control measures should be incorporated into house design and management and documented by periodic monitoring.
Common Risk Reduction Practices in Salmonella Control Programs (continued)

- Rigidly enforced biosecurity practices should be implemented to restrict the movement of personnel and equipment onto poultry housing premises and between houses.
- Feed should be pelleted or contain no animal proteins.
- Water should come from sources that are treated to ensure microbiologic purity.
Changes in Human S. Enteritidis Incidence in 31 States after Implementation of Egg Quality Assurance Programs

- Decreased
- Increased

Source: CDC
Flock management and sanitation practices can proactively attack the sources of *Salmonella* infection in poultry flocks and their environment rather than merely identifying infected flocks or rendering flocks less susceptible to infection. However, this is a long-term approach to risk reduction and may not provide a rapid response for problems with severe short-term public health or economic consequences. Moreover, attention to environmental issues does not directly address vertically transmitted *Salmonella*. 
Conclusions

No single response (serotype-specific or serotype-independent) is likely to be an effective unilateral solution to the complex public health and economic problems associated with *Salmonella* in poultry.

Comprehensive quality-assurance programs, incorporating both the coordinated and sustained implementation of risk-reduction practices throughout the production continuum and targeted testing to detect pathogens of concern, have yielded promising results in several nations.
Conclusions (continued)

Although most quality-assurance programs emphasize risk reduction, testing provides essential verification of the efficacy and cost-effectiveness of risk reduction practices (and identifies flocks infected with uniquely problematic serotypes).

Preventive treatments such as colonization control or vaccination can reduce the *Salmonella* susceptibility of poultry in case risk reduction practices fail to prevent pathogen introduction into flocks. Vaccination also enhances the short-term responsiveness of control programs to address problems associated with specific serotypes of elevated significance.
Regulations for Shell Egg Producers in the USA (Implemented July, 2010)

- Implement a written *S. Enteritidis* prevention plan.
- Maintain rodent/pest control and biosecurity programs.
- Purchase chicks from uninfected (NPIP-certified) breeder flocks and raise pullets under *S. Enteritidis*-monitored conditions (with environmental testing at 14-16 weeks of age).
- Test laying house environmental samples for *S. Enteritidis* at 40-45 weeks of age and 4-6 weeks after induced molting.
- If environmental testing is positive for *S. Enteritidis*, clean/disinfect thoroughly between flocks and test eggs. If egg testing is positive for *S. Enteritidis*, divert eggs for pasteurization.
- Store and transport eggs under refrigeration at 45°F (7.2°C) beginning 36 hours after laying. Eggs may be equilibrated to room temperature for an additional 36 hours prior to processing.
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“The only thing that makes life possible is permanent, intolerable uncertainty, not knowing what comes next.”

---Ursula K. LeGuin---