**Introduction: Reducing *Salmonella* Enteritidis contamination of shell eggs**

P. H. Patterson,*2 K. Venkitanarayanan,† and S. Kariyawasam‡

*Department of Animal Science, Pennsylvania State University, University Park, 16802; †Department of Animal Science, University of Connecticut, Storrs 06269; and ‡Department of Veterinary and Biomedical Science, Pennsylvania State University, University Park 16802

**Primary Audience:** Egg Industry Personnel, Food Scientists, Veterinarians, Poultry Scientists

**SUMMARY**

*Salmonella* Enteritidis is a leading food-borne pathogen in the United States, with many outbreaks in humans traced back to shell eggs. As a result, the implementation of effective strategies for reducing *Salmonella* Enteritidis in commercial layer flocks has become a critical public health and economic objective. In this paper, we share the findings of 2 multistate USDA-National Integrated Food Safety Initiative grant teams and their work aimed at *Salmonella* Enteritidis reduction in shell eggs. One project, led by K. Venkitanarayanan, is using plant-derived antimicrobial molecules as dietary supplements to reduce *Salmonella* Enteritidis colonization of the digestive and reproductive tract of chickens. The same molecules are being evaluated for their effect on *Salmonella* Enteritidis in egg wash solutions. The project led by S. Kariyawasam used on-farm investigation and novel bacterial typing methods to study *Salmonella* Enteritidis transmission in diverse layer environments to update and optimize Egg Quality Assurance Programs that will significantly reduce *Salmonella* Enteritidis contamination of shell eggs. The current US Food and Drug Administration Egg Safety Rule and Egg Quality Assurance Programs are based on critical control points and best management practices developed from studies of large flocks (>50,000 hens) conducted in the 1990s, indicating that opportunities exist to improve preharvest programs to reduce *Salmonella* Enteritidis contamination. This paper will share the findings of these 2 projects.

**Key words:** *Salmonella* Enteritidis, egg, foodborne illness, egg quality assurance program

http://dx.doi.org/10.3382/japr.2014-00940

**DESCRIPTION OF PROBLEM**

The poultry industry is under increasing consumer and regulatory pressure to guarantee food safety and meet export requirements. Surveillance systems for food-borne diseases, such as FoodNet (Food-borne Diseases Active Surveillance Network), generate a report card for food safety that captures disease trends over time. Highlights from the 2012 data, which monitored...
15% of the United States population, identified 19,531 laboratory-confirmed cases of foodborne infection [1]. Salmonella infection was up 3% from previous years, yet not significantly compared with 2006 to 2008 data. In 2012, 16.4 Salmonella cases were reported per 100,000 people; however, this is still greater than the national goal of 11.4 or less cases per 100,000 established by the Centers for Disease Control [1]. According to the USDA-Food Safety Inspection Service (FSIS), Salmonella in meat and poultry products is the most pressing problem they face and causes an estimated 1.3 million illnesses every year [2]. In December of 2013, FSIS released their “Salmonella Action Plan,” which included several aggressive steps the agency will take to prevent Salmonella-related illnesses.

Salmonella Enteritis is a leading foodborne pathogen in the United States and one of the most common serovars associated with foodborne illness. The Food and Drug Administration (FDA) estimates that 142,000 illnesses each year are caused by consuming eggs contaminated with Salmonella Enteritidis [3]. Salmonella Enteritidis in egg layers emerged as a significant issue in the United States after an epidemic in the northeastern region in the 1980s was traced back to contaminated eggs [4–6]. Since that time, Salmonella Enteritidis has spread to flocks throughout the United States, causing numerous egg-related outbreaks and food-borne disease [7–11]. For example, in 2010, the largest outbreak of salmonellosis in United States’ history was traced back to eggs produced by layer flocks in the state of Iowa [12].

Salmonella Enteritidis is the most frequently isolated Salmonella from layer flocks [13–16]. The primary colonization site of Salmonella Enteritidis in chickens is the cecum [17–19], leading to horizontal transmission into the environment, contamination of eggshell with feces, and likely retro-contamination of ovaries [20]. Contamination of egg contents with Salmonella Enteritidis results by penetration through the eggshell from contaminated feces during or after oviposition [21–26]. Horizontal egg contamination with Salmonella Enteritidis can also occur through environmental sources, such as farmers, pets, and rodents [27]. Following oviposition, Salmonella Enteritidis survival or growth on the shell surface of eggs is supported by the presence of chicken manure and other moist organic materials [20].

Contamination of egg contents (yolk, albumen, and eggshell membranes) with Salmonella Enteritidis can also occur by direct transmission of the pathogen from infected hen ovaries or oviduct by transovarian route (vertical transmission) before oviposition [28–33]. Gantois et al. [20] reported that Salmonella Enteritidis has the ability to persist in the reproductive organs of hens, despite the immune response mounted by the birds. Several researchers believe that internal contamination of eggs with Salmonella Enteritidis is the outcome of pathogen colonization in the hen reproductive organs [34–36]. Most often, systemic colonization of birds with Salmonella results in the pathogen spreading to the reproductive organs [37–39]. Uptake of Salmonella by hen macrophages after bacterial invasion of intestinal cells is believed to disseminate the pathogen within the host, including the reproductive organs [29–31, 40, 41]. In addition to systemic spread, Salmonella Enteritidis colonization of the hen reproductive tract can also result from an ascending infection from the cloaca [29, 42] or a descending infection (retro-contamination) from the ovary [34]. Salmonella Enteritidis can colonize many sites in the hen reproductive tract, including the ovary, infundibulum, magnum, isthmus, and vagina, each resulting in the contamination of the egg [20].

Preharvest intervention strategies are significant not only because of the capacity of Salmonella Enteritidis for vertical transmission from the hen to the eggs, but also horizontal transmission from contaminated feces, insects, rodents, litter, water, and feed to layers on a farm. Infection of chickens after oral ingestion of Salmonella Enteritidis from environmental sources results in systemic spread and subsequent colonization of the ovary and oviduct [43, 44]. Due to the multitude of Salmonella Enteritidis sources, implementation of strict biosecurity measures at the farm and efforts aimed at reducing the level of Salmonella Enteritidis in the gastrointestinal tracts of birds are critical to reducing the spread of the pathogen.

Public health agencies and shell egg producers continue to develop standards and control
strategies aimed at eliminating or reducing enteric pathogens in the food chain, including Salmonella Enteritidis in shell eggs [4, 5, 45–52]. The National Poultry Improvement Plan has developed guidelines under the Salmonella Enteritidis Clean Program to verify breeder flocks are Salmonella Enteritidis-free [53, 54]. In addition, certain states have developed Egg Quality Assurance Programs (EQAP) that rely on hazard analysis critical control point (HACCP) practices to reduce the risk of Salmonella Enteritidis infection in layers and eggs. Pennsylvania was the first state in the United States to develop an EQAP [4, 5, 46, 55, 56]. This program, known as the Pennsylvania Egg Quality Assurance Program (PEQAP), was established in 1994 and was based on the research of the 1992 USDA Salmonella Enteritidis Pilot Project. The PEQAP has greatly reduced Salmonella Enteritidis prevalence in commercial layers within Pennsylvania and has been a model HACCP-based food safety program for EQAP throughout the United States [4, 5, 46, 55, 57]. Salmonella Enteritidis prevalence is very low in states such as Pennsylvania, which have efficient EQAP, compared with states with no equivalent [57]. In 1992, before implementation of PEQAP, 38% of flocks, 23% of environmental samples, and 0.026% of eggs were positive for Salmonella Enteritidis on contaminated farms in Pennsylvania [4, 5, 46]. A significant decline in these numbers was noted after implementation of PEQAP, and, in 2008, only 7.4% of flocks and 1.6% of samples were diagnosed Salmonella Enteritidis-positive [4, 5, 46]. The situation further improved, and by 2011 only 1.85% of flocks, 0.29% of samples, and 0.011% of eggs on contaminated farms were positive for Salmonella Enteritidis [58].

However, due to the lack of a decline in Salmonella Enteritidis outbreaks in humans nationally, and because eggs are the most common source of infection, the FDA recently issued the “Final Egg Rule” to prevent Salmonella Enteritidis in shell eggs during production, storage, and transportation [59]. Large flocks (>50,000 birds) have been obligated to comply with the rule since July 2010, and medium-sized flocks (3,000–50,000 birds) since July 2012 [60]. However, producers with small flocks of less than 3,000 birds are currently exempt from this rule. Furthermore, the FDA egg rule was largely based on PEQAP, with most of the research and Salmonella Enteritidis-monitoring studies conducted in the early 1990s and focused on large production facilities (greater than 50,000 hens) [46–48, 55, 56]. As a result, it is unknown what level of risk medium- and small-sized farms (especially farms with less than 3,000 birds, backyard flocks, and multiple species farms, for example, farms with cattle, hogs, horses, and so on) may pose to public health. Eggs from these farms may well pose a higher risk due to the fact that these producers have fewer technical and financial resources at their disposal. Management systems encountered in small egg-producing flocks are often quite diverse, including multiple types of cage-free systems such as pastured poultry, organic, aviary systems, and enriched cages. Eggs are often marketed via direct retail to restaurants, health-food stores, farmers markets, and on-farm stores. Because of these diverse management and marketing practices, it is unknown whether EQAP developed with large producers in mind are entirely applicable to medium- and small-sized flocks. Given that poultry management practices have changed significantly since the inception of PEQAP in 1994, the exact routes of Salmonella Enteritidis movement still remain largely unknown in alternative production settings. Because of the possible higher risks posed by small- and medium-sized farm settings, it is now critical to revisit EQAP to trace routes of Salmonella Enteritidis transmission throughout the entire egg system to accurately identify CCP and thus update and optimize an EQAP for all producers throughout the United States to prevent Salmonella Enteritidis contamination of shell eggs.

**TWO USDA-NATIONAL INTEGRATED FOOD SAFETY INITIATIVE PROJECTS**

Two project teams integrating research and extension are directly addressing the USDA priority of filling knowledge gaps about sources and persistence of pathogens associated with eggs and applying control measures for reducing these pathogens.
Reducing Egg-Borne Outbreaks of Salmonellosis Using Plant-Derived Antimicrobials

Principal investigator Kumar Venkitanarayanan enlisted co-investigators Mike Darre (University of Connecticut), Paul Patterson, Patricia Curtis (Auburn University, Auburn, AL), and Honwei Xin (Iowa State University, Ames, IA) for this study. The objectives of the study were to (1) determine the effect of trans-cinnamaldehyde, carvacrol, thymol, and eugenol as dietary supplements to reduce colonization of Salmonella Enteritidis in the intestinal and reproductive tracts and eggs of laying hens, and to determine their efficacy as an antimicrobial wash for shell eggs; (2) to determine if consumers can detect eggs from hens fed trans-cinnamaldehyde, carvacrol, thymol, and eugenol or washed with these plant compounds compared with standard eggs; and (3) develop and implement outreach programs to educate egg producers or processors about these antimicrobials as part of a total EQAP to reduced Salmonella in or on eggs.

Development of an Updated, Optimized EQAP to Minimize Salmonella Enteritidis Contamination of Shell Eggs

Principal investigator Subhashnie Kariyawasam enlisted co-investigators Paul Patterson, Eva Pendleton (Pennsylvania State University), Ed Dudley (Pennsylvania State University), Stephen Knabel (Pennsylvania State University), and Darrell Trampel (Iowa State University). The overall goal of this project was to develop an updated and optimized EQAP that will significantly reduce Salmonella Enteritidis contamination of shell eggs throughout the United States. Specific aims of this study were to (1) determine the routes by which Salmonella Enteritidis is transmitted to shell eggs in the layer environment of flocks with 3,000 to 50,000 hens (medium) or less than 3,000 hens (small), (2) determine the effect of flock size and vaccination on Salmonella Enteritidis contamination, (3) establish new CCP and an updated optimized EQAP, (4) evaluate the effectiveness of the EQAP before and after it is optimized, and (5) disseminate the optimized EQAP for industry, government, and academics to implement.

CONCLUSIONS AND APPLICATIONS

1. Salmonella Enteritidis is still a leading food-borne pathogen, with many outbreaks in the United States traced back to the consumption of shell eggs. Implementation of effective strategies for reducing Salmonella Enteritidis in commercial flocks has become a critical public health and economic objective. Because little progress has been made reducing Salmonella Enteritidis outbreaks nationally, and because eggs are the most common source of infection, the FDA issued the Final Egg Rule, which was implemented in 2 phases for large- (2010) and medium-sized (2012) farms to prevent Salmonella Enteritidis in shell eggs. The USDA-FSIS recently released their Salmonella Action Plan, with aggressive steps the agency will take to prevent Salmonella-related illnesses from meat and poultry products.

2. Two project teams integrating research and extension are addressing the knowledge gaps about sources and persistence of Salmonella Enteritidis associated with eggs and applying control measures for reducing these pathogens. The titles of these 2 projects are Reducing Egg-Borne Outbreaks of Salmonellosis Using Plant Derived Antimicrobial, and Development of an Updated, Optimized Egg Quality Assurance Program to Minimize Salmonella Enteritidis Contamination of Shell Eggs.

3. The findings from these 2 projects were reported at the symposium Reducing Salmonella Enteritidis Contamination of Shell Eggs with an Integrated Research and Outreach Approach sponsored by the Poultry Science Association at their 2013 annual meeting in San Diego, California. Herein are the journal articles from the symposium covering the progress of these projects and significant findings to date to reduce Salmonella Enteritidis contamination of shell eggs.

REFERENCES AND NOTES

PATTERSON ET AL.: SYMPOSIUM: REDUCING SALMONELLA


52. United Egg Producers. 2001. UEP “5-STAR” total quality assurance program: A HACCP type food safety program with validation. UEP, Atlanta, GA.


**Acknowledgments**

The authors greatly appreciate the funding from the USDA-National Integrated Food Safety Initiative to support the work reported herein.