Evaluation of egg safety begins when the egg is laid. To conduct a thorough risk analysis the risk assessment must begin at lay and continue through to consumption of the egg by the consumer. The goal of this publication is to provide guidance in evaluating risk from farm to consumer. The risk analysis guidance will provide some research results that will aid in risk analysis for each production and processing step. The layers, production environment, egg temperature (initial and throughout processing and storage), and wash water pH and temperature play key roles in reducing microbial growth in shell eggs and should be key in developing an egg safety plan. We have compiled data from published research related to the shell egg production and processing environment, which could be helpful when developing an egg safety plan. Board and Tranter noted that shell eggs can acquire bacteria from every surface they contact (Board and Tranter 1995). Unlike our earlier publication on this topic, this publication includes the layer house and the processing area. It is important to consider all risks associated with the egg. Many of today’s operations are inline and, therefore, a true shell egg risk assessment should evaluate the risks associated with the egg production as well as the egg processing areas. However, with the shift to more off-line and cage-free production systems, a thorough risk assessment should be done for the entire production through processing.

*Salmonella Enteritidis (SE)* is an organism of concern for the layer industry. Contamination of eggs can occur either vertically (infected ovaries) or horizontally (penetration through the shell).
Denagamage et al. conducted a systematic review of observational studies on risk factors associated with *Salmonella* in laying farms. They found the presence of previous *Salmonella* infection, absence of cleaning and disinfection, presence of rodents, induced molting, larger flock size (>30,000 hens), multiage management, cage housing systems, in-line egg processing, rearing pullets on the floor, pests with access to feed prior to movement to the feed trough, visitors allowed in the layer houses, and trucks near farms and air inlets were identified as the risk factors associated with *Salmonella* contamination in laying hen premises. A high level of manure contamination, middle and late phase production, a high degree of egg-handling equipment contamination, a flock size greater than 30,000, and an egg production rate greater than 96% were identified as risk factors associated with *Salmonella* contamination of shell eggs (Denagamage, et al. 2015).

**Cleaning and disinfection of layer house:**

In 1995, Jones et al. conducted a study where they found *Salmonella* in 72% of the samples collected from the layer house environment (Jones, Rives and Carey 1995). In 2013, a U.S. research project found that only 1% of environmental samples from layer houses tested positive (Animal and Plant Health Inspection Service [APHIS] 2014). Castellan et al. found manure drag swabs useful for validating SE control programs (Castellan et al. 2004). In a study conducted by Garber et al., none of the layer houses tested positive for SE on farms where the feeders and hoppers were cleaned and disinfected between flocks. Also, none of the houses tested positive where cages, walls, and ceilings were washed between flocks, whether or not they were fumigated. They also reported that a reduced risk of SE was not identified in their study when cages and walls were dry cleaned or when egg belts and elevators were cleaned (Garber et al. 2003).

**Rodents and other pests: (Castellan, et al. 2004)**

It is common knowledge that house flies carry bacteria. A 2008 study found that contaminated hens can rapidly infect flies. Layers eating contaminated flies can also become infected (Ausmus 2008).

Wild birds and mammals are generally regarded as the main reservoir for *Salmonella* in the environment. Warm-blooded animals can carry *Salmonella* in their intestinal tract without showing any signs of illness (Meerburg and Kijlstra 2007). These animals can then transfer the *Salmonella* to the environment and the hens.
In 2003, Garber et al. reported that the prevalence of SE in mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses. A 2013 U.S. study found that farms that tested positive for SE had a moderate or high rodent index, routinely molted birds, and had a downtime of 10 days or less (Animal and Plant Health Inspection Service [APHIS] 2014).

**Molting:**

Induced molting is a practice that has been used since the 1930s to rejuvenate laying flocks for a second or third cycle of production. Several molting methods have been studied over the years, including 1) feed removal or limitation, 2) low nutrient rations, and 3) various feed-additives (Bell 2003). Based on numerous research studies, the commercial egg industry over the past 50-plus years has generally used a total feed withdrawal of up to 14 days with water supplied ad libitum to *initiate the molt*. Over the past 10 to 15 years, however, the animal rights and animal welfare communities have become increasingly critical of the total feed withdrawal practice for inducing the molt. Thus, the industry has been looking for alternative procedures for molting flocks (United Egg Producers 2002). One potential procedure that has been looked at in the past is the maintenance of birds on a very low density (i.e., low protein, low energy, high fiber) diet during the normal feed withdrawal part of the molt (Anderson and Havenstein 2007).

Piao et al. studied the impact of SE vaccinated and unvaccinated hens subjected to an SE challenge and various feed withdrawals on SE-infected eggs and organs. They found more SE-positive eggs in the unvaccinated group than in the vaccinated group of hens, suggesting that SE vaccination of pullets may protect against SE infection during forced molting (Piao, et al. 2007). APHIS reported that the post-molt period presents a higher risk for presence of SE compared with other stages of production (Animal and Plant Health Inspection Service [APHIS] 2014).

The decision whether to molt or not seldom rests with the integrated egg producer. A successful molting program requires close cooperation between the production and marketing segments of the firm. At no point is this cooperation more important than at the laying house. A variety of non-anorexic induced molting methods are used today. AG-801, *Induced Molting of Commercial Layers* ([https://content.ces.ncsu.edu/induced-molting-of-commercial-layers](https://content.ces.ncsu.edu/induced-molting-of-commercial-layers)) outlines the most successful molting techniques available.

**Multiage flocks:**

Denagamage et al. reported the risk of SE was greater with multi-age flocks (Denagamage, et al. 2015).

**Housing systems:**

Garber et al. reported that flocks that had been primarily floor-reared as pullets were 5.9 times more likely to test positive for SE than were flocks that had been cage-reared (Garber, et al. 2003).
A UK study reported SE persisted in houses longest (>15 months) in step-cage and cage-scraper houses when high levels of rodents were present, and for the shortest duration in non-cage and cage-belt houses. The UK study also reported a greater persistence of SE than non-SE serovars when the rodent score was 1.5 (“1” = few rodent signs, “2” = moderate rodent signs, “3” = high level of rodent signs) (Carrique-Mas, et al. 2009).

Castellan et al. (2004) reported that the use of watering systems that store water, such as cups and troughs, were more strongly associated with SE than systems that utilize nipple dispensers. (Castellan, et al. 2004)

### Biosecurity:

Egg safety regulations require a biosecurity program include limiting visitors on the farm and in poultry houses; maintaining personnel and equipment practices that will protect against cross-contamination from one poultry house to another; preventing straying poultry, wild birds, cats, and other animals from entering poultry houses; and prohibiting employees from keeping birds at home (21 CFR 118.4(b)).

### On Farm Egg Storage:

The FDA’s Egg Safety Final Rule requires that eggs are held and transported at or below 45°F ambient temperature beginning 36 hours after the time of lay. For off-line egg production, that means eggs must reach the processing facility within 36 hours of lay or the farm must have an on-farm cooler that meets this requirement.

### Control Measures:

APHIS recommends controlling rodents, having a downtime greater than 10 days, and vaccinating pullets to reduce the risk of SE occurrence on table-egg farms (Animal and Plant Health Inspection Service [APHIS] 2014).

### Testing Procedures:

**Rodent Testing**

Egg rule guidance for industry (Food & Drug Administration 2011) states that as part of the producer’s pest control program, a producer must monitor for rodents by visual inspection and mechanical traps, glueboards, or another appropriate monitoring method. When monitoring indicates unacceptable rodent activity within a poultry house, use appropriate methods to achieve satisfactory control.
One method described in the FDA guidance document for monitoring rodents is rodent indexing. The Rodent Index (RI) is based on the total number of rodents caught in a house in 7 days using 12 live catch traps and is used to estimate the rodent population. FDA recommends the following procedure for obtaining the RI:

- **Bait each of 12 live-catch rodent traps with 0.5 oz. of chicken feed.**
- **Place the traps in areas where recent signs of rodent activity are observed or in areas most likely to catch rodents (e.g., along cage walkways and against walls.)** Use a minimum 15-feet distance between traps.
- **Check the traps after 2 to 4 days. Remove, count, and record the number of rodents caught.**
- **Move the traps that did not catch any rodents to a different location, a minimum of 15 feet away. Traps that caught a rodent are placed back in the same location.**
- **Check the traps again 7 days after they were first placed.**
- **Record the total number of rodents caught for the week.**

The following formula standardizes all rodent catches to a 1-week period using 12 live-catch traps by adjusting for periods of time longer or shorter than 7 days and using more or fewer traps.

\[
\text{Number of rodents for RI} = \left( \frac{\text{total number of rodents in traps}}{\text{working traps set}} \right) \times 12 \times \frac{7}{\text{number of days traps are set}}
\]

The rodent population is then estimated by applying the number of rodents determined with the formula above to the RI table below. An RI of 1 or less is likely to indicate satisfactory rodent control. If an RI greater than 1 is obtained, the producer should investigate to find out where rodents are entering the house. Section 118.4(c)(1) requires that appropriate methods be taken to achieve satisfactory rodent control when monitoring indicates unacceptable rodent activity within a poultry house.
**Environmental Testing**

Environmental testing should be conducted for SE when laying hens are 40 to 45 weeks of age and 4 to 6 weeks after molt. FDA’s recommended sampling and detection methods can be found on the [Environmental Sampling and Detection of Salmonella in Poultry Houses](#) web page.

**Egg Sampling**

Egg testing is usually a last resort. Trying to locate the egg(s) that might contain *Salmonella* in a layer house is like trying to find a needle in a haystack. You will need to collect a lot of eggs to find the potentially contaminated egg. Even a layer that is positive for SE only sometimes lays contaminated eggs. Therefore, eggs are collected and pooled for sample testing. The regulatory requirement for egg testing begins 10 days after notification of a positive environmental test. Eggs from a flock in a house that has tested environmentally positive for SE legally may continue to be marketed as table eggs until the producer is notified that an egg test is determined positive for SE. Once an egg sample has tested positive for SE, those eggs must be diverted for treatment.

The video in Figure 1 demonstrates the approach that should be used to collect eggs that will be submitted for SE testing.

<table>
<thead>
<tr>
<th>Number of rodents caught in 7 days with 12 traps</th>
<th>RI</th>
<th>Description of RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>11-25</td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>26 or more</td>
<td>3</td>
<td>High</td>
</tr>
</tbody>
</table>

![Figure 1. Egg sampling methodology (CLICK TO VIEW VIDEO)](image)
**Processing Plants:**

**Incoming eggs:**

The Egg Safety Final Rule requires that eggs are held and transported at or below 45°F ambient temperature beginning 36 hours after the time of lay. For off-line egg production, that means that eggs must reach the processing facility within 36 hours of lay or that the egg was stored in an on-farm cooler that met this requirement. It is important to know the internal temperature of the eggs entering the washer. If eggs are more than 18°F colder than the wash water, they will need to be tempered prior to washing to reduce the risk of shell breakage (Weasley and Beane, 1967). If the eggs come from an in-line source, it is important to know their temperature because the wash water should be 20°F warmer than the warmest egg.

Cooling eggs prior to washing raises concerns regarding the potential for sweat to form on the eggs. This concern is based on the assumption that sweat provides the water needed for bacteria to penetrate the shell and contaminate the egg. However, a recently completed study did not support this finding. Gradl et al. reported that sweating did not increase contamination of bacteria through the shell (Gradl, et al. 2017).

**Pre-processing egg storage**

Although most eggs are produced by large in-line operations, some are still produced from off-line sites, and this number is expected to increase. In off-line processing plants (where eggs are brought from off-premises) initial internal egg temperatures of 41 to 45°F (5 to 7.2 °C) are likely. Coolers for unprocessed eggs should maintain an ambient temperature of 45°F (7.2 °C) or lower (USDA Agricultural Marketing Service 2000). Although pre-processing coolers are held generally between
41 to 45 °F, egg temperatures decline only slightly. However, tempering of the eggs prior to washing is important to minimize thermal checking. Egg temperatures generally range from 88 to 96°F (31.1 to 35.6 °C) when they reach the processing area.

**Equipment and facilities**

Separate areas should be provided for the storage of packing and packaging materials. Chemical compounds should be kept in a secure area to prevent potential damage to the containers and to restrict access to the individuals in charge of application. Insecticides and rodenticides should be maintained in areas away from the processing area (USDA Agricultural Marketing Service 2000).

Musgrove et al. (2012) reported that nest run egg carts may serve as reservoirs for *Salmonella* in the shell egg-processing environment (Musgrove, Shaw and Harrison 2012).

**Pre-wash**

USDA regulations state that eggs cannot be immersed at any time but may be pre-wet prior to washing if sprayed with a continuous flow of water – of similar temperature to that of the wash water – that drains away.

**Egg washing**

While washing shell eggs is not allowed in the European Union (EU), it is allowed or even encouraged in Canada, Australia, Japan, and the U.S. The reluctance of washing in the EU appears to be based on research conducted in the early part of the 20th century (Hutchison, et al. 2003). The problem with using these early studies to determine whether to wash eggs today is that the eggs in the early studies were immersion washed using varying wash water temperatures. Immersion washing is no longer allowed. Spray washing does not have the same negative impact as immersion washing.

Cleaning eggs during washing is related to wash water temperature, water quality characteristics (i.e., hardness, pH), detergent type and concentration, and defoamer. Replacement water in washer tanks should be added continuously to maintain a constant overflow rate, according to USDA regulations. Chlorine or quaternary ammonium sanitizing compounds may be used as part of replacement water, provided they are compatible with the detergent. Only potable water with an iron content of less than 2 ppm may be used to wash eggs (USDA Agricultural Marketing Service 2000). Rate and extent of bacterial growth during storage is favored by washing eggs in water with less than 2 ppm iron, so it is important to monitor the iron content of the wash water. USDA suggests that water with an iron content in excess of two
ppm should not be used unless deironized (Baker and Bruce, 1994). Iron contamination may influence microbial growth if shell membranes are compromised or penetrated. As bacteria grow on membranes in an iron-rich environment, they can produce metabolic products that allow microorganisms to penetrate and diffuse into the albumen, providing a more favorable medium for microorganism growth.

Egg temperature at processing is critical. USDA regulations require that wash water temperature be 90°F or higher, or at least 20°F warmer than the highest egg temperature (whichever is greater). These temperatures must be maintained throughout the cleaning cycle. Temperature of incoming eggs will vary from season to season and from operation to operation. Regulations also require that wash water be changed every four hours, or more if needed, to maintain sanitary conditions and that replacement wash water be added continuously to maintain a continuous overflow (9 CFR 290.515). When the difference between wash water temperature is greater 40 °F, thermal checks and cracks increase, allowing surface microbes greater access to the interior of the egg. Contact between wash water and eggs during processing causes internal egg temperatures to increase. Although blow-drying following washing slightly decreases internal egg temperatures, they generally rise throughout the process and can continue to rise for up to six hours after eggs are placed in a cooler.

Most processors use wash water much hotter than the minimum 90°F. A survey by Anderson et al. (Anderson, Jones and Curtis, Heat Loss from Commercially Packed Eggs in Post-Processing Coolers 1992) found North Carolina processors use wash water temperatures that range from 115 to 120°F. In 1955, Hillerman reported that wash water maintained at 115°F would increase the internal egg temperature by 0.4 °F/second (Hillerman 1955).

Detergents and detergent dispensers must be listed as approved for use on eggs in the current List of Propriety Substances and Nonfood Compounds, Miscellaneous Publication Number 1419 (U.S. Department of Agriculture Food Safety and Inspection Service 1985). The best approach for reducing microbial populations in wash water tanks and, therefore, on eggs occurs when detergents are added in amounts sufficient to maintain a pH of 11.

Alkaline cleaning formulations produce an initial pH near 11 in the wash water, and the pH during operation usually continues in the 10-11 range, unfavorable for growth of most bacteria (Moats 1978). However, Jones et al. isolated S. heidelberg from the shell of a commercial egg processed in water when the pH fell below 10.2 (Jones, Rives and Carey 1995). Two Canadian researchers, Holley and Proulx (1986), found Salmonella species could survive at 38 and 42°C (100.4 and 107.6°F) when wash water pH was less than or equal to 9.5 (Holley and Proulx 1986). Alkaline pH has been reported to increase the sensitivity of Salmonella to heat (Anellis, Lubas and Rayman 1954) (Cotterill 1968). Kinner and Moats found that at pH 10 and 11, bacterial counts decreased regardless of water temperature. They also reported that bacterial counts decreased at 50 and 55°C (122 and 131°F) regardless of pH (Kinner and Moats 1981). Laird et al. indicated, however, that
current processing practices are not sufficient to prevent potential contamination of washed eggs with *Listeria monocytogenes*. Their study showed that *Listeria* is isolated readily from egg processing environments, including wash water (Laird, Bartlett and McKellar 1991). Pearson et al. reported that egg wash water with a high pH was bactericidal to *E. coli* and *Salmonella* and recommended that HACCP programs include regular sampling and analysis (Pearson, Southam and Holley 1987). Several research studies have shown that a pH of 10 or 11 or above is necessary to control bacteria. pH is a relatively inexpensive variable to monitor, and doing so offers significant protection against such bacteria as *Salmonella Enteritidis*.

Many shell egg processors have no idea about the variation in pH in their wash water. Often, those who monitor measure pH only at the start of a shift. pH may be 10 or 11 at the beginning of the shift, but recycling wash water, overflow losses, and added replacement water all contribute to reducing pH levels. Detergents elevate the pH of egg washers and are dispensed, for the most part, in concentrations necessary to clean the egg shell. As a result, minimal thought is given to maintaining a constant pH. Dual tank systems present special problems, as the pH in each tank can be different, depending on how the wash systems are connected.

The Food Production and Inspection (FPI) Branch of Agriculture Canada monitors egg-grading stations routinely in Canada to ensure that recirculating types of egg washers maintain the following wash water conditions: a minimum wash water temperature of 40°C (104°F), a minimum pH of 10, and a wash water result of 100,000/g aerobic colony count (ACC) or less (Canadian Food Inspection Agency 2013). These conditions were established to eliminate pathogens that may be present in the wash water and to minimize microbial contamination of the washed eggs. At present, bacterial numbers in egg wash water are monitored to ensure that adequate sanitation is achieved. Total viable counts greater than 105 cfu/ml are considered unacceptable (Bartlett et al, 1993). The U.S. currently has the only regulations governing wash water temperature and time between water changes in the tank.

Defoamers also play an important role in egg washing. When defoamers are not dispensed properly, foam in the wash tanks eventually builds up and overflows. When foam spills from the tanks, it can interfere with water level detection and affect water temperature and pH.

Washing, drying, and candling unit operations are generally continuous operations. Eggs detected as "dirties" at candling must not be soaked in water for cleaning, because soaking in water for as little as 1 to 3 minutes can facilitate microbial penetration through the egg’s shell.

Egg washing, sanitizing and oiling should always be conducted according to the procedures outlined in the current *Regulations Governing the Voluntary Grading of Shell Eggs* [7 CFR Part 56].

Processing rooms and all equipment should also be thoroughly cleaned at the end of each processing day.
**Rinse and dry:**

Eggs should be rinsed by spraying with water slightly warmer than the wash water. An approved sanitizer should be used in the spray rinse to remove any remaining bacteria. The strength of the sanitizing spray should be no less than 50 ppm and no more than 200 ppm of available chlorine or its equivalent (USDA Agricultural Marketing Service 2000). After washing, the hot water rinse may contain chlorine or quaternary sanitizers that are compatible with the washing compound. Iodine sanitizing rinse may not be used as part of the replacement water. Ambient air under high velocity dries the eggs. At this point the surface temperature of the egg reaches approximately 95°F. Anderson et al. found that the internal temperature of eggs continues to rise due to high shell surface temperatures and candling lights. Five minutes after the eggs were processed their temperature was 12 to 14 degrees above their initial temperature (Anderson, Jones and Curtis, Heat Loss from Commercially Packed Eggs in Post-Processing Coolers 1992).

Shell eggs may be oiled after drying, provided operations are conducted in a manner to avoid contamination of the product. Previously used processing oil that has become contaminated can be filtered and heat-treated at 180°F for three minutes prior to reuse.

**Post Processing Storage:**

Eggs are packaged in cartons or flats, placed in cases, and cases are palletized. Efficient packaging procedures such as these ensure that high egg temperatures due to processing will be maintained for several days. In fact, recent industry surveys by the authors suggest as much as a week is required to dissipate temperature increases from processing under these conditions. Yet virtually everyone in the shell egg processing industry uses these or similar packaging procedures. Federal law requires processed egg coolers maintain an ambient air temperature of 45°F (7.2°C) or lower and a relative humidity of 70 to 85 percent (USDA Agricultural Marketing Service 2000).

Researchers have found that the growth rate of *S. Enteritidis* in eggs responds directly to the temperature at which the eggs were stored, and that holding eggs at 40 to 45°F reduced the heat resistance of *Salmonella Enteritidis* (Denagamage, et al. 2015). It has been suggested that refrigeration reduces the level of microbial multiplication in shell eggs and lowers the temperature at which the organism is killed during cooking. This, in and of itself, may be adequate justification to store eggs at 40 to 45°F.

Humidity in the storage environment is important both in maintaining egg weight and preventing microbial growth. A relative humidity of 60% during storage can cause weight loss and a corresponding increase in air cell size. However, storage in a relative humidity of 80% can promote microbial growth.
**Control Measures:**

Controlling egg temperature is crucial for controlling SE growth. Therefore, monitoring egg temperatures is recommended. The pH level of wash water in recirculating eggs washers is key to controlling SE growth in the recycled water, and the pH levels should be monitored throughout the processing period. Eggs should be cooled as soon as possible after washing and packaging. Although the internal egg temperature will most likely not reach 40°F prior to departure from the facility, eggs should be quick-stored in a cooler and maintained in refrigerated conditions throughout their shelf life.

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