Toward Better Control of *Salmonella* Contamination by Taking Advantage of the Egg's Self-Defense System: A Review

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Abstract: Egg-associated salmonellosis is a major problem for food safety. It can be caused by vertical transmission (transovarian transmission) in hens and horizontal transmission though penetration. Despite a series of physical and chemical defense mechanisms naturally found in eggs, they cannot provide complete protection for them. Environmental hygiene, bacteria vectors such as birds, rodent, flies, and beetles along with feed and water contamination are the most frequently reported causes of *Salmonella* colonization in hens, and finally to eggs. In addition, inappropriate egg handling will cause eggs to lose their self-protection ability, thus resulting in the survival and multiplication of *Salmonella* in an egg's contents, which contributes to the horizontal dissemination. The routes of *Salmonella* contamination were discussed, and the effectiveness and shortcomings of different decontamination methods were evaluated in this review. Various studies on egg storage indicated that the low-temperature storage without temperature fluctuation was beneficial for the control of *Salmonella*. This review, based on an understanding of the stages of *Salmonella* transmission and an egg's self-protection mechanisms, highlights a comprehensive strategy toward *Salmonella* control in a process from egg production and handling to human consumption.

Keywords: egg safety, egg's self-protection mechanism, *Salmonella* control, *Salmonella* transmission

Introduction

In the United States, 164044 *Salmonella* infections were reported during 1998 to 2002 (Lynch and others 2006). In China, a total of 4207 *Salmonella* Enteritidis infections from egg products were reported from 1991 to 1996 (Liu 2008). The latest egg-associated *Salmonella* outbreaks, which happened in the United States from May to August in 2010, led to a nationwide recall of more than half a billion eggs from 2 Iowa egg producers, and nearly 2752 illnesses were reported from May to September 2010. This caused egg safety to come into the spotlight of the food industry once again.

Salmonella is a genus of rod-shaped, Gram-negative, nonspore forming, thermolabile, predominantly motile enterobacteria with diameters of approximately 0.7 to 1.5 μm, and lengths from 2 to 5 μm. Most *Salmonella* have flagella with the exception of *Salmonella* Gallinarum and *Salmonella* Pullorum. Flagella and curli fimbriae are vital for penetration through an egg's physical barriers where multiplication can occur in the nutrient-rich yolk. The most commonly isolated *Salmonella* serovars from poultry and eggs were *Salmonella enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis (Omwandho and Kubota 2010). However, *S.* Enteritidis phage type $(PT)_4$ was more frequently reported than other *S.* Enteritidis phage types (Cogan and others 2004).

Salmonella is a food borne bacteria causing human infection, which often results from the consumption of uncooked contaminated eggs or infected egg products. A level of *Salmonella* contamination of 10 to 20 CFU per egg was sufficient to cause human salmonellosis (Kapperud and others 1990; Vought and Tatini 1998). Although humans, regardless of their age or sex, were vulnerable to *Salmonella* contamination, the most vulnerable were children under the age of 5, the elderly, and those with impaired immune systems. Symptoms developed 8 h to 3 d after eating contaminated food and last 4 to 7 d with characteristics including diarrhea, fever, nausea, stomach cramps, vomiting, and headache (Lin and others 1997; FSIS 2005).

In artificially infected hens, the percentage of infected eggs could range from 0% to 27.5% (De Reu and others 2008). In naturally infected situations, the detection rate varied according the seriousness of contamination in flocks. For example, in the United Kingdom, there was a *Salmonella* isolation rate of 3.4% of 17000 eggs sampled between 2002 and 2004 (Little and others 2007), while the estimation from the World Health Organization showed that 0.03% of the eggs were contaminated with *S.* Enteritidis in infected flocks in Israel. The average for more than 60% of the flocks was only 0.025% (Lublin and Sela 2008). Even though the *Salmonella* detection rate is relatively low in most cases, the problem is still serious if we take the whole shell egg consumption amount into consideration. According to the risk assessment conducted by USDA-FSIS, 46.8 billion eggs were produced annually in the United States, and 2.3 million of them might contain *Salmonella*. Those eggs contaminated with *Salmonella* pose a great risk to public health without proper handling and processing.

A better understanding of the interactions and mechanisms between *Salmonella* and chicken eggs is necessary to reduce outbreaks of salmonellosis. Considerable research has been reported on egg components in terms of bacteria contamination examples include the quality of the cuticle and eggshell, components of the egg

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white and their functions, and factors that affect the penetration of *Salmonella* and its resistance abilities. Additional research has been reported on how to control *Salmonella* infection in table eggs more effectively. In this review, all items were summarized from the whole picture.

Two Routes for Eggs to Be Contaminated with *Salmonella*

There are 2 possible routes for egg contamination by *Salmonella*. Eggs can be contaminated by *Salmonella* originating from the infection of reproductive organs though direct contamination of the yolk, albumen, eggshell membranes, or eggshells before oviposition. Second, eggs can also be contaminated by penetration through the eggshell from the colonized gut or from contaminated faeces during or after oviposition. In this case, many different serotypes of the genus *Salmonella* can be involved (FEHD 2004). However, it is uncertain that route is most important for egg contents to become contaminated by *Salmonella* Entertidis.

Vertical transmission

In the case of vertical transmission, *S.* Enteritidis could be introduced into the egg from infected ovaries or oviduct tissue before oviposition (Gantois and others, 2009). When *Salmonella* are directly deposited inside the egg contents of an egg, they are able to survive especially in egg yolk, which is a favorable environment for *Salmonella* multiplication. Once the bacteria colonized in the hen's intestinal tract, it could invade and disseminate to internal organs and maintain colonization throughout a whole production period (Gast 1994; Gast and Holt 1998). Based on the strain and the dose of the inoculum, flocks infected with *S.* Enteritidis showed various clinical symptoms, such as depression, anorexia, diarrhea, reduced egg production, and even mortality as well as physiological effects like different levels of antibodies in egg yolk and serum (Shivaprasad and others 1990; Gast 1994). Infected flocks excreted *S.* Enteritidis intermittently (Shivaprasad and others 1990). Carrique-Mas and others (2008) revealed that only 5% of the birds from the infected flocks actively excreted *Salmonella* at the end of lay and lower in earlier stages. Eggs from those infected birds could be internally contaminated in intermittent clusters (Humphrey and others 1989). Further experiments showed that short environmental stress, such as water and food deprivation, an infection with other pathogens, and molting, were often the common catalysts leading to an increase of *Salmonella* excretion in flocks (Barrow 1992; Nakamura and others 1994). However, no certain correlation was found between persistence of *Salmonella* in flocks and the likelihood of egg contamination (Gast and others 2005a). And according to FSIS (2005), most cases of foodborne salmonellosis in the United States were associated with the consumption of shell eggs, and the predominant *Salmonella* were transferred by vertical transmission.

Horizontal transmission

Environment hygiene is rather critical for the control of *Salmonella* dissemination in horizontal transmission. Carrique-Mas and others (2009) revealed that *Salmonella* could exist in laying houses over subsequent flock cycles therefore posing a persistent threat to the poultry industry. *Salmonella* existing in laying house is able to penetrate though the eggshell and vitelline membrane into egg contents and consequently infects humans.

Penetration through eggshell. The eggshell is composed of a cuticle and mammillary, cone, palisade, and vertical crystal layers. The eggshell has pores that allow the exchange of gas and water between its contents and the external environment. Therefore, the pores also provide a passageway for bacteria in the environment to enter the egg. Accordingly, an understanding of the influence of eggshell quality and *Salmonella* penetration is necessary for taking measures to prevent the introduction of *Salmonella* into eggs via penetration of the eggshell. Penetration is facilitated mainly by condensation that occurs as the egg passes through the vagina and experiences a temperature change from that of the hen to the outside environment. Eggshell characteristics changed with the age of the hen with its best overall qualities during the middle of the egg production (Messens and others 2005a). However, there was no correlation between eggshell characteristics, such as the number of pores or thickness and *Salmonella* penetration (Kraft and others 1958; Williams and others 1968; Nascimento and others 1992; Messens and others 2005a). And current evident showed that bacteria were checked in their movement by their structural modifications in their mammillary layer (Messens and others 2005b). On the other hand, some extrinsic factors, such as strain of bacteria, temperature differential, moisture, number of organisms present, and storage conditions, were identified as being important to the trans-shell contamination (Messens and others 2005b).

Penetration through vitelline membrane. The vitelline membrane surrounding the egg yolk is made up of 2 layers. The outer layer consists of the ovomucin, vitelline membrane outer layer protein I (VMO I), VMO II, and lysozyme. The inner layer consists mainly of glycoprotein I(GP I), GP II, and GP III (Kido and others 1975, 1992). Bacteria penetration through the vitelline membrane can result in rapid and extensive multiplication in the nutrient-rich yolk content. *In vitro* experiments, which involved inoculating *S.* Enteritidis and *Salmonella* Heidelberg onto the exterior surface of egg yolk vitelline membranes, showed that bacteria may sometimes reach the yolk (Gast and others 2005b).

Leleu's study (2009) showed that vitelline membrane strength (VMS) decreased from the beginning of a production cycle to the middle of it and then plateaued until the end of the production cycle. Comparisons of VMS during different periods of lay and between the VMS and the moment of penetration by *Salmonella* revealed little effect of the lay period and a slight but significant correlation between the VMS at the moment of penetration by *Salmonella*. Thus, it can be assumed that a higher VMS leads to a longer resistance of the vitelline membrane against penetration. Interesting, however, was that lower yolk temperatures resulted in higher VMS (Ngoka and others 1983). This partly explains why low-temperature storage reduces the risk of *Salmonella* vitelline membrane penetration. Other studies showed that VMS decreased with storage (Kirunda and McKee 2000; Jones and Musgrove 2005), which was partly a result of loss of structural integrity (Fromm 1966; Back and others 1982).

Self-Defense Mechanism of Chicken Eggs

An egg is a potential life form having its own protection mechanisms. Thus, the intact egg is naturally equipped with both physical and chemical defenses in order to protect the embryo from bacteria invasion and physical harm. This is shown in the following aspects.

Physical barriers

Cuticle. Oviposited eggs have a cuticle deposition, which covers the outer surface of the eggshell. The cuticle is composed of a protein carbohydrate complex and contains a small amount of the crystal complex hydroxyapatit. This layer is secreted in the shell gland pouch during the last hour of eggshell formation (EFSA

2009). The cuticle is the first line of defense to bacteria by closing the eggshell pores and decreases with the age of the hen.

Eggshell. The eggshell has many pores, which can vary in number from 6000 to 10000 according to egg size and location. There are more pores on the blunt pole than at the apex and some of the proteins related to the eggshell have antibacterial properties. The pore diameters range from 6 to 65 μm and are wide enough for the penetration of *Salmonella*.

Eggshell membrane. The inner and outer eggshell membrane can act as a "filter" in the process of penetration from external sources. The eggshell membrane is composed of highly cross-linked proteins that are structurally similar to a meshwork of entangled threads and can obstruct the invading microorganisms (Baker and Balch 1962). Ahlborn and others (2006) reported that bacteria thermal resistance and/or their inactivated cells were greatly reduced by exposing the selected Gram-positive and Gram-negative bacterial pathogens to eggshell membranes. This process is associated with several antibacterial proteins, such as ß-N-acetylglucosaminidase, lysozyme, and ovotransferrin.

Chemical barriers

Bactericidal effects of albumen were factors that influence *S.* Enteritidis's growth in egg albumen with the restriction of iron being a major factor (Schade and Caroline 1944; Clay and Board 1991; Baron and others 1997). A high concentration of ovotransferrin in egg albumen could chelate iron and inhibit bacterial growth by binding free iron and making it inaccessible to bacteria resulting in a depletion of ferric and ferrous iron for *Salmonella*. Moreover, ovotransferrin and lysozyme could interact with the surface of *S.* Enteritidis and form pores in the *Salmonella* cell wall, thus preventing its multiplication (Kang and others 2006). Also, egg albumen could penetrate *S.* Enteritidis and kill bacteria through nuclease activity (Lu and others 2003).

Genes involved in cell wall structural and functional integrity, as well as nucleic and amino acid metabolism were important for *S.* Enteritidis to persist in egg albumen (Clavijo and others 2006). Therefore, damaging bacterial DNA became one of the mechanisms that egg albumen uses to control bacteria growth. This might be because egg albumen nuclease could damage the chromosomal DNA of *Salmonella* by entering bacterial cells through pores in the cell wall formed by ovotransferrin and lysozyme. In addition, there were other factors that work against *Salmonella* such as immunoglobulins in egg albumen and yolk, the albumen's alkaline pH, protease inhibitors, proteins chelating essential elements, and the viscosity of albumen that prevents *Salmonella*'s movement into the egg's yolk. These bactericide effects not only play an important role on horizontal transmission, but also limit the growth of *Salmonella* in the eggs contents in the case of vertical transmission (Keller and others 1995).

Controlling *Salmonella* Contamination in Eggs and Egg Products

Reasons that eggs are vulnerable to *Salmonella* contamination

Although an egg has many self-defense mechanisms against bacterial invasion, *Salmonella* also set up systems to avoid defenses by the egg albumen. For instance, *yaf*D and *xth*A were genes that play an essential role in the repair of DNA damage cause by the albumen and hence facilitate the survival of *S.* Enteritidis in chicken eggs (Lu and others 2003; Gantois and others 2009). The survival ability for *Salmonella* in egg contents also depends on the strain of *Salmonella* and preservation temperature. Many studies indicate that *S.* Enteritidis can survive in egg content (Gast and Holt 2000; Messens and others 2004; Murase and others 2005; Gurtler and Conner 2009). However, there was no agreement about *Salmonella* growth in albumen because it is difficult to compare various studies due to different inoculum size, strains, incubation temperatures, storage time, and age of the eggs. Gast and Holt (2000) indicated that extensive multiplication of *S.* Enteritidis was less frequently observed at lower inoculum doses (15 cells), shorter storage times (1 d), and lower temperatures (10 to 17.5 °C) (Gast and Holt 2000). *Salmonella* Enteritidis inoculated onto a vitelline membrane could proliferate in albumen surrounding the yolk, possibly resulting from the use of nutrient compounds emerging from the yolk though the vitelline membrane (Murase and others 2005). However, no such indication was found that nutrients or factors leaking out from the yolk could impair the inhibitory properties of the albumen. And growth of *Salmonella* occurred more frequently in the albumen of fresh eggs compared to eggs stored prior to inoculation, which was partly due to the alkalinity pH environment in egg albumen since longer periods of storage results in higher albumen pH (Scott and Silversides 2000; Messens and others 2004). In Gurtler and Conner's study (2009), survival of *Salmonella* in liquid egg products was also related to storage temperature and egg product composition. Humphrey and Whitehead (1993) found that *S.* Enteritidis can grow in the contents of naturally contaminated eggs at room temperature. Besides, the effectiveness of an egg's self-protection abilities was affected in the case of inappropriate preservation. *Salmonella* can double every 20 min and a single bacterium can multiply into more than a million in 6 h if not properly handled (Hasan and others 2009). Multiplication of *Salmonella* in eggs could occur rapidly within a single day of storage at a warm temperature. Study showed that the number of *S.* Enteritidis in yolk can reach a mean level of 8.4 to 8.7 log units/mL at 2 d in samples initially contaminated with doses of 15 and 150 CFU, respectively, while a mean level of 4.3 log units/mL (15 CFU dose) and 6.1 log units/mL (150 CFU dose) at 2 d in whole egg. No multiplication was found in albumen (Gast and Holt 2000). Therefore, concerns on how to reduce egg contamination to the greatest extent through better treatment and handling of fresh eggs is a rather important issue for public health.

Comprehensive Measures toward *Salmonella* Control in Flocks

The primary reason for eggs getting contaminated with *Salmonella* is the infection of flocks. To the best of our knowledge, numerous factors can result in the colonization of *Salmonella* in hens such as feed, water, vectors, as well as general hygiene condition. According to FDA investigation, the *Salmonella* outbreak that happened at the Wright County Egg farms in the United States was possibly due to feed contamination and other common environmental risk factors such as equipment, walkways, and other surfaces in and around the farm (CDC 2010). Given the seriousness of this issue, it is urgent for egg producers to strictly implement preventive measures throughout the whole egg production process. First of all, a timely disposal of waste and dead birds limits the number of vectors on farms. The hygiene procedure including a thorough cleaning of flock housing followed by an effective disinfection of the flock surface should be carried out consistently. For another, feeds as a common source of *Salmonella* for poultry flocks, should always be decontaminated with heat treatment, and processed in pellet form rather than in meal form to lower the flock's risk of *Salmonella* contamination. In some cases, when *Salmonella* infection becomes a serious problem, a special control strategy such as the use of a vaccine is necessary to prevent *Salmonella* colonization in the reproductive tract, as well as to reduce fecal shedding and further contamination of eggs. Two types of vaccines are available for poultry at present and both of them can be used throughout the life of birds except during the withdrawal period before slaughter. However, the use of a vaccine does not provide 100% protection in flocks. And it has limited effects on improving animal health and welfare. Therefore, its use depends on the aim of the control program, type of poultry, stage of production, true prevalence of *Salmonella*, serovars targeted, detection methods used, and cost-benefit analysis (EFSA 2004). In addition, an integrated control program for *Salmonella* should include the "all-in-all-out" principle and "test-and-removal of flock policy." Since it is unrealistic for the complete eradication of *Salmonella*, comprehensive bio-security measures should be adopted constantly.

Egg handling and processing

Decontamination. Because egg decontamination effectively reduces the bacteria load on an eggs surface and prevents rapid penetration, it should be carried out as soon as eggs are collected. Ways to decontaminate eggs include different detergents in wash water, such as free chlorine, new N-halamine compounds, and an iodine-based disinfectant, (Worley and others 1992; Knape and others 2001), electrolyzed oxidative water (Russell 2003), microwave (Mudau 2007; Sivaramakrishnan 2007), ultrasonic in combination with heat and/or pressure treatment (Piyasena and others 2003; Cabeza and others 2004, 2005), ozone, and UV (ultraviolet radiation) (Rodríguez Romo 2004). The use of egg washing is a continuous debate despite its broad commercial application. Current concerns focus on whether egg washing increases the internal microbial load. Within the European Union, egg washing is prohibited except in Sweden and parts of the Netherlands. The reason offered is that egg-washing procedures may damage the quality of the cuticle enhancing the opportunity for bacterial invasion (Peebles and Brake 1986; Bialka and others 2004; EFSA 2005). Factors related to cuticle damage caused by egg washing include presence of water on the eggshell, presence of iron in the wash water, physical brushing damage, and high pressure (Commission of European Communities, 2003). These are the reasons that class A eggs for human consumption are not eligible for the practice of egg washing by European Union legislation and eggs will be downgraded if any forms of disinfection are used. However, this reasoning is at odds with research that showed the washing procedure did not appear to affect the incidence of open pores and the overall cuticle quality. Meanwhile, it was also indicated that brown eggs in general were of better quality in terms of their cuticle scores than the white eggs when 4 standards, such as mechanical damage, debris, open pores, and cuticle coverage, were considered (Messens 2009). And the use of egg washing is yet authorized in Canada, America, Japan, Australia, Russia, and Mexico for the reason that egg washing can reduce the total microbial load on the surface of sanitized eggs by approximately 2 to above 5 log units (Hutchison and others 2004; Rodríguez Romo 2004).

Given the controversy on the advantages and disadvantages of egg washing, other procedures are being evaluated. Hierro and others (2009) used pulsed light (PL) as a method of egg decontamination and the effects were more notable when the cuticle was preserved intact. In addition, this treatment is most effective when applied as soon as eggs are laid. And, it is limited by the motility of *Salmonella* and low penetration depth of UV radiation. Hot air treatment for table eggs (2 shots for 8 s at 600 °C with an interval of 30 s of cold air) reduced *Salmonella* load up to 1.9 log units without significant changes for any of the egg's quality traits, such as the cuticle, breaking strength, and yolk index (Pasquali 2009). Nonthermal atmospheric gas plasma device, a resistive barrier discharge prototype able to generate an ionized gas containing free electrons and neutral reactive species such as atoms, molecules, and radicals at atmospheric conditions, which is able to reduce *Salmonella* load up to 4.5 log units per eggshell with a humidity of 65% at 25 °C for 90 min of treatment, provides a decontamination choice for farmers and industries that need stock eggs for a relatively long period (Ragni and others 2010). However, the time and cost needed for this method may become a limitation for its practical use in commercial production.

In hot water immersion, heat is transferred from hot water through the eggshell all the way to the inside egg contents until the center of the yolk reaches the desired temperature for sufficient time. In light of early research findings, the use of hot water immersion at 57 °C for 25 min followed by hot air heating at 55 °C for 60 min resulted in a 7 log unit reduction of *Salmonella* in shell eggs and produced acceptable changes on egg qualities at the same time (Hou and others 1996). The patent of Davidson and others (2004) indicated that the heated fluid bath with a temperature of between about 128 to 145 °F allows a reduction of at least 4.6 log units of any *Salmonella* bacteria within the eggs. This followed by antibacterial gas treatment and further wax cover could result in at least another 5 log units reduction of bacteria and provide additional antibacterial barriers to egg contents. And, this method is commercially used by Davidson's pasteurized eggs. Nevertheless, pasteurization methods employing liquid immersion or spray washing of shell eggs are prohibited under certain regulatory schemes in many European countries due to possible undesirable effects to egg quality (Ball and others 2002). Schuman and others (1997) revealed that 50 to 57.5 min treatment with a bath temperature of 58 °C or 65 to 75 min treatment with a temperature of 57 °C increased Haugh unit values and had no influence on albumen pH values and yolk index but that it affected albumen clarity and functionality.

In addition to *Salmonella* disinfection, an effective measure for preventing *Salmonella* growth in egg contents is necessary. The rapid cooling was introduced to cool eggs from 40 or 45 °C to 7 °C in approximately 15 min or less, which may take 7 or 10 d in conventional conditions, to suppress the significant bacteria multiplication (Keener and others 2000b). Moreover, rapid cooling was also found to improve internal egg quality and increase shelf life (Sabliov and others 2002). Further study showed that rapid cooling with CO_2 produces higher quality eggs with increased shelf life than rapid air cooling but with no difference on Haugh units unless followed by subsequent storage in CO_2 (Keener and others 2000a). Although rapid cooling might cause slight cracks in eggshells, if well managed, it is a good way for controlling *Salmonella* growth on the whole (Thompson and Knutson 2000).

Most importantly, though lots of work can be done for shell eggs, secondary pollution in the process of packing and palletizing may also introduce *Salmonella* to decontaminated eggs. Thus, decontamination should not only focus on the egg itself, but the equipment for egg storage as well.

Storage. Storage conditions present issues in contamination with focus on duration, temperature, and environmental hygiene. Different countries have different regulations. Storage limits for table eggs in the United Kingdom were 3 wk at 8 °C (Kinderlerer 1994), while in Israel 3 mo for refrigerated eggs and 16 d at room temperature (Lublin and Sela 2008). In many countries, eggs are required to be stored at low temperatures to restrict microbial growth. In Germany, legislation required that egg cooling be applied at 5 to 8 °C for 18 d maximum post lay (EFSA 2009). And in the United States, either shell eggs packed for consumers or eggs that receive a treatment from egg producers were required to be kept at 45 °F (7.2 °C) no later than 36 h after the eggs are laid during storage and transportation (FDA 2010). In this scenario, it is more advisable to apply low-temperature storage in order to minimize the possibility that eggs infected with *S.* Enteritidis are transmitted to humans. This recommendation is supported by the study of Gast and Holt (2000), which showed that low temperatures were more effective for controlling *S.* Enteritidis multiplication in the yolk when high concentration of *S.* Enteritidis was artificially introduced into egg contents. (Gast and Holt 2000). On the other hand, low temperature can slow down the process of penetration (Chousalkar and others2010). However, Kang and others (2006) suggested that it is preferable to store eggs at 37 °C for a certain period of time first, instead of 4 °C directly, to allow the endogenous bactericidal activity of egg albumen to kill the contaminating *S.* Enteritidis. This reasoning is valid especially when most eggs are infected through trans-shell contamination. While in the case of vertical transmission, this application awaits more research. Further studies show that, although low preservation temperature for table eggs will limit the multiplication of *Salmonella*, it does not reduce the existing *Salmonella* concentration. It may indeed prolong the survival of *Salmonella* because *Salmonella* may be increased by low storage temperature (Baker and Balch 1962; Radkowski 2002; Messens and others 2006) and reduced with higher temperature (Rizk and others 1996).

From the aspect of an egg's structure, Humphrey and Whitehead (1993) showed that storage had little direct impact on albumen with respect to the growth of *Salmonella*, but rather, it influenced the integrity of the vitelline membrane that might result in a difference in *Salmonella* multiplication in the albumen and yolk. The pH of the albumen rose along with the storage time, and finally led to the dissipation of fibers contained on the vitelline membrane of fresh eggs, as well as the decline in protein and hexosamine content of the vitelline membrane (Fromm 1967). Consequently, it is easier for the bacteria to either invade the yolk or obtain nutrients from it. Therefore, cooling practices should to be carried out shortly after lay to keep eggs fresh and also to prevent *Salmonella* multiplication in eggs. Besides, the speed with which changes in membrane integrity occur as during storage, it is also highly temperature dependent. No significant changes occurred over 3 wk of storage at 20 °C, whereas apparent changes occurred only after 7 to 10 d of storage with temperatures fluctuating between 18 and 30 °C (Humphrey and Whitehead 1993). Thus, it is highly recommended that eggs should be kept in a cooling environment and temperature fluctuation be avoided during egg storage.

Additional concern for egg processing. Eggshell is especially fragile so special care should be taken in the process of egg handling. The data by USDA showed that 177 million dozen shell eggs were cracked during August 2010, 3% up from a year ago (USDA 2010). A cracked egg loses part of its defense system, thereby is in danger of *Salmonella* invasion. Cross-contamination, on the other hand, always happens in transportation and egg processing. Therefore, safety education for egg handlers is necessary to mitigate *Salmonella* dissemination and egg products should be pasteurized. It was estimated that the annual number of illness would be reduced from 5500 to 3200, if all liquid egg products produced in the United States were pasteurized for a 6 log units reduction of *Salmonella* (FSIS 2005). Outreach efforts should stress the importance of properly cooking eggs. *Salmonella* are susceptible to heat treatment. Temperatures above 55 °C for enough time are sufficient to destroy *Salmonella*. Uncooked and semi-cooked eggs should be avoided for the public.

Conclusion

An egg can be contaminated either though vertical transmission or horizontal transmission. A well-organized *Salmonella* control scenario is necessary in virtue of the seriousness of *Salmonella* dissemination. First of all, flocks are the primary contamination resource for eggs. Thereby, egg producers have to keep good environmental hygiene, ensure feed and water safety, and implement effective management strategies to guard hens against *Salmonella* infection. On the other hand, decontamination and storage conditions turn out to be rather critical in the process of egg handling. A number of decontamination methods were evaluated in the review. Egg handlers have to make a proper choice in light of their practical values coupled with specific regulations and cost-benefit analysis. When it comes to storage condition, low-temperature storage is basically more favorable and should be carried out as soon as possible after eggs are laid. In this scenario, rapid cooling can shorten the cooling time to prevent multiplication of *Salmonella*. Moreover, temperature fluctuation and long time storage should be avoided to keep the integrity of the vitelline membrane thus retarding the growth of *Salmonella* in egg contents. Most importantly, eggs have to be well cooked for enough time in case *Salmonella* recover from pasteurization.

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