

1 **ECOLOGY OF *SALMONELLA* AND MICROBIAL CONTAMINATION IN POSTHARVEST**

2 **TABLE SHELL EGG PRODUCTION**

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Abstract

Egg production and processing continue to be sources of foodborne *Salmonella* spp. that can lead to foodborne illnesses. *S. Enteritidis* remains one of the primary serovars associated with laying hens that can end up in the table shell eggs. There are several potential routes of *Salmonella* contamination both during egg production in the layer house as well as egg processing and beyond. This review briefly discusses preharvest aspects of *Salmonella* infection in laying hens and subsequent contamination of eggs. Most of the focus in this review will be on postharvest ecological and environmental aspects of *Salmonella* and general microbial table shell egg contamination occurring during collection, processing and retail. Current interventions as well as potential opportunities for additional efforts to limit *Salmonella* and microbial contamination in shell eggs during processing will also be discussed.

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1.0 Introduction

Foodborne disease linked to *Salmonella* spp. continues to be problematic in the United States public health sector with extensive economic consequences and continued need for regulatory action (Scallan et al., 2011; Ricke et al., 2013d). Although foodborne pathogen *Salmonella* spp. can originate from a variety of food sources, poultry meat and table eggs remain prominent sources (Suslow et al. 2003; Foley et al., 2008, 2011, 2013; Hanning, et al., 2009; Erickson, 2010; Foley et al., 2011, 2013; Carrasco et al., 2012; Finstad et al., 2012; Howard et al., 2012; Koo et al. 2012; Painter et al., 2013). Several *Salmonella* serovars have been identified in outbreaks of human disease with *Salmonella enterica* serovar Typhimurium along with *S. Enteritidis*, *S. Newport* and *S. Heidelberg* being the ones most likely to occur as the causative isolate (Finstad et al., 2012; Foley et al., 2013).

For table shell eggs and the associated food products formulated with egg components *S. Enteritidis* has been the serovar that is usually identified as the primary foodborne disease causing serovar (Patrick et al., 2004; Braden et al., 2006; Curtis, 2007; Finstad et al, 2012; Howard et al., 2012; Martelli & Davies, 2012). Historically, *S. Enteritidis* emerged as primarily an egg related serovar that remained persistent in egg production cycles and could be identified with major salmonellosis outbreaks associated with *Salmonella* (St. Louis et al., 1988; Cogan & Humphrey, 2003; Martelli & Davies, 2012; Ricke et al., 2013a). This may in part be due to the ability of *S. Enteritidis* to gravitate toward the physiological processes that are exclusive to the generation of eggs by laying hens (Guard-Petter, 2001; Ricke, 2003a; Curtis, 2007; Gantois et al., 2009; Howard et al., 2012; Martelli & Davies, 2012; Galiş et al. 2013).

96 Consequently considerable effort has been made to document and describe the role that
97 preharvest egg production plays in generation of contaminated eggs and how this impacts
98 commercial egg production. Postharvest control measures for reducing *S. Enteritidis*
99 contaminated eggs have also been documented to some extent. However, as alternative egg
100 production systems and local egg marketing expands commercially in the U.S. some of these
101 issues will need to be examined with restrictions unique to those systems in mind. Likewise as
102 more emphasis is placed on sustainability and recycling this will potentially influence food
103 safety of eggs. Finally, as international commercial egg production expands and opens new local
104 markets egg safety may need to also be an issue under these particular production systems and
105 retail circumstances. Therefore, the current review will focus on particular aspects of egg
106 processing, carton packaging and handling in retail both in current production practices as well
107 as historical developments and future considerations as retail production practices and associated
108 markets become more variable.

109 **2.0 Historical and current trends in commercial egg production**

110 Since 2011 the average table layer hen numbers in the United States has continued to
111 expand from 281.6 million layers in 2011 to 296.4 million layers as of Dec. 1, 2013 based on
112 United States Department of Agriculture (USDA) estimates (Ibarburu & Bell, 2013). These
113 recent flock numbers are reflective of the tremendous changes in the U.S. egg industry that have
114 occurred over the past century where increases in the size of flocks have followed technological
115 changes in layer building efficiency with single buildings housing thousands of birds and farm
116 complexes capable of managing hundreds of thousands of birds (Parkhurst & Mountney, 1988;
117 Klippen, 1990; Dunn & Madison, 1992; Bell, 1993; Anderson, 2009). Implementation of
118 technologies such as mechanical egg gathering systems for in-line collection of eggs contributed

119 to this expansion of numbers of birds per flock and house (Bell et al., 2001). Likewise,
120 innovations in egg processing plants for candling, egg washing, and packaging made further
121 expansion possible (Klippen, 1990; Kuney et al., 1995; Hutchison et al., 2003; Musgrove, 2011).
122 Consequently, the layer farms and number of flocks decreased while the number of birds per
123 farm and per flock increased (Bell et al., 2001). This higher egg supply capacity from fewer
124 flocks and farms and development of much more efficient packing plants along with the market
125 impact of uneven regional changes in U.S. human population growth predictably led to industry
126 wide interregional relocation shifts (Dunn & Madison, 1992; Bell 1993; Ryan et al., 1996).
127 Regional differences in the U.S. were detectable not only from a pricing and product selection
128 standpoint but the type of packaging, variety of products and egg quality (Bell et al., 2001;
129 Koelkebeck et al., 2001).

130 Similar trends have occurred internationally as countries become more developed, their
131 corresponding agricultural industries begin to expand and adjust to advanced technologies. This
132 led to shifts in international markets which were extensively discussed elsewhere by Windhorst,
133 (2006, 2008a,b, 2009) as a series of market analyses reviews and will not be discussed in detail
134 in the current review. The following is a brief overview of these key conclusions previously
135 made by Windhorst. For roughly three and half decades, global poultry meat and egg production
136 outpaced beef, veal and swine (Windhorst, 2006). During this time shifts occurred as egg
137 production volume in developing countries surpassed production in developed countries and by
138 2005 over 60 % originated from the Asian continent and nearly half came from three countries,
139 China, India, and Japan with China producing over 40% and exhibiting tremendous growth
140 during this time (Windhorst, 2006, 2008a, 2009). Just from 1990 to 2007 alone, nearly a
141 doubling in global egg production occurred (Windhorst, 2009). However, the vast majority of

142 these eggs have remained in domestic markets with relatively few of the total eggs produced
143 being exported and imported (Windhorst, 2006). Part of this may be due to the existence in
144 some of these developing countries of small family flocks that are raised for subsistence
145 consumption and local sales (McLeod et al., 2009). The global egg trade that does occur is
146 highly regional with over two thirds of the exports and imports occurring in Europe with other
147 regional trade zones centered in the American and Asian continents (Windhorst, 2008a,b,2009).

148 Since the early to mid 2000's several factors have come into play to once again shift the
149 U.S. and international egg industry not only in terms of how eggs were marketed but how they
150 were produced on commercial farms. Specialty egg brands that included eggs originating from
151 organic and welfare managed production systems, nutritionally modified eggs, fertile eggs and
152 eggs produced from hens fed all vegetable diets emerged in retail markets as viable, albeit more
153 expensive options for customers (Patterson et al., 2001; Jones et al., 2010). Early interest in
154 generating eggs that were considered more compatible with cholesterol reduction diets in
155 humans led to hens fed vegetarian diets and commercially produced eggs containing higher
156 levels of omega 3- fatty acids (Michella & Slaugh, 2000). As public health demands grew
157 further, developments in fortifying egg nutritional value by increasing polyunsaturated fatty acid
158 content, vitamin A, D and E by specific dietary strategies and feeding regimes of laying hens
159 became an ongoing focal point (Rossi et al., 2013). Since the egg composition is feed dependent,
160 this trend of dietary manipulation to further alter eggs to fit human nutritional and health needs is
161 anticipated to continue as more understanding is achieved on the physiology and metabolism of
162 egg formation (Shapira, 2010).

163 Animal welfare and subsequent consumer concerns have also impacted both the U.S. as
164 well as the international egg industry. These concerns led the European Union to ban

165 conventional cage housing in 1999 with the idea of replacing them with alternative housing
166 systems by 2012 (De Reu et al., 2008; Mench et al., 2012). These alternative housing systems
167 consisted of either furnished cages that included a nest box, a dust bath, and a perch, or fully
168 non-cage based systems (De Reu et al., 2008). Non-cage systems contain nest boxes and floor
169 litter areas and are referred to either as aviaries which consist of some sort of platforms and
170 slatted floors at different heights or if at one height simply called floor housing and when
171 allowing access to outdoors can be considered free range (De Reu et al., 2008). Holt et al.
172 (2011) concluded that as these regulations are implemented and alternative housing operations
173 expand in commercial egg production systems, the impact on egg microbiology and pathogen
174 prevalence will need to be assessed. Historically, these types of systems were in place in the
175 mid-1900's and some of this information could be potentially gleaned from studies conducted at
176 that time. However, Anderson (2009) points out that this would be problematic as much of the
177 management data available from these earlier flock systems would not be applicable because the
178 breeds and other factors were different than they are in current operations.

179 Most of the more recent microbiology studies have been conducted in Europe (Jones et
180 al., 2011) and Holt et al., (2011) noted that these results were conflicting, with some concluding
181 that conventional cage operations yielded a greater opportunity for *S. Enteritidis* incidence while
182 others suggesting that nonconventional gave higher incidences of *S. Enteritidis*. Numerous
183 confounding factors probably play a role in these different outcomes including the impact of
184 flock size, type and frequency of sampling, seasonality, layer breed, management differences,
185 and stress response, just to name a few (Curtis, 2007; De Reu et al., 2008, 2009; Anderson et al.,
186 2009; Dewulf et al., 2011; Holt et al., 2011; Jones et al., 2011). Clearly more work needs to be
187 done to not only characterize microbial contamination levels in these operations on a wider

188 scale, but develop analyses that can identify and account for the multiple factors which may be
189 different among egg layer operations. Once this is accomplished, management practices and
190 intervention strategies can be designed to be precisely applied to particular types of operations
191 for more effective control of *Salmonella* incidence. In addition more effort may need to be
192 directed toward detailed epidemiology of *Salmonella* isolates in these systems to avoid potential
193 differences in serovars that could also confound control strategies.

194 The other major factor that is emerging in egg production and processing systems that
195 potentially will impact egg microbiology is assessment and implementation of improved
196 sustainability. Sustainability of agriculture in general has become an issue as energy costs both
197 for production of cereal grains used as feed stocks and maintaining livestock housing have gone
198 up while public concerns over environmental impact of confined animal operations have
199 increased as well. For global egg production, Windhorst (2007) raised this question as a function
200 of whether grain-fed layer flocks would be economically compromised as the arable land for
201 corn and oil seed crops become more diverted into bioethanol and biodiesel production in
202 different countries including the U.S. Increased interest in egg production sustainability also
203 derives from other venues. Certainly, as discussed previously, the development and spread of
204 alternative layer housing raises the issue of balancing animal welfare versus economics as a
205 function of sustainable costs. Likewise, the emerging consumer driven market for organically
206 produced poultry and animal products with the associated restrictions in grain production and
207 management options has raised the question as to the sustainability of such operations for future
208 expansion into more traditional agriculture markets (Ricke et al. 2012b). Organically produced
209 eggs are also on the rise (van der Sluis, 2007; Anderson, 2009) so it is anticipated that similar
210 questions will be raised for these types of egg production systems as well.

211 Sustainability of confined animal operations and impact on environment are closely
212 linked. For egg production systems Xin et al. (2011) reviewed and compared environmental
213 impacts between conventional caged housing systems and nonconventional systems and
214 concluded that noncage houses generally have poorer air quality due to ammonia and dust and
215 are generally less energy, feed and land efficient. They suggested that research was needed to
216 compare the overall carbon footprint among the different housing systems to design means to
217 reduce the environmental impact using life cycle assessment tools. Life cycle assessment (LCA)
218 have been utilized to quantitate sustainability in a number of agriculture systems. Essentially
219 LCA analyses involves constructing a balance sheet for all environmental inputs and outputs
220 involved in the production, transportation, distribution, disposal and any other steps associated
221 with the entire life cycle of a product (Guinée et al., 2011). Recently, several LCA studies have
222 been made on egg production systems. Pelletier et al. (2014) used LCA to demonstrate that the
223 overall environmental footprint for the U.S. egg industry was reduced in 2010 compared to 1960
224 and that the three primary environmental impacts during this time span were feed efficiency,
225 feed composition, and manure management. Leinonen et al. (2012) using LCA to quantify
226 environmental impact per kg eggs produced from cage, barn, free range, and organic United
227 Kingdom (U.K.) production systems, concluded that organic eggs required the most hens and
228 cage systems the least number of hens, with organic hens consuming the most feed. They also
229 noted that feed production, processing and transportation elicited the largest impacts on the
230 environmental footprint. Suggestions for further reducing the greenhouse gas environmental
231 footprint in free range chickens have included substituting protein sources for the cereal grains
232 currently used in these diets (Taylor et al., 2014).

233 In summary, several drivers are in play that could potentially be changing the dynamics
234 of commercial egg operations on an international scale. As more efforts are made to change
235 housing systems to address animal welfare concerns, and local free range and organic production
236 system markets continue to increase, much of what is known about the prevalence of
237 microorganisms in general and in particular *Salmonella* spp. may no longer apply in these
238 modified systems. Likewise, further attempts to reduce the environmental footprint by altering
239 feed composition and the handling of manure and other waste material could also alter the
240 transmission patterns of the organisms. This may in turn have large impacts on egg food safety
241 as international markets and regional production systems continue to shift. Consequently, it will
242 be critical to examine what is already known about the ecology of *Salmonella* in egg production
243 and processing and use this as starting point to revisit and in some cases identify potential critical
244 control points that may be unique to these systems. The following sections will discuss
245 *Salmonella* ecology in egg production and processing and examine current intervention
246 strategies.

247 **3.0 Egg production management on the farm and incidence of *Salmonella***

248 There are numerous opportunities for laying hens or for that matter chickens in
249 commercial production systems in general to come in contact with *Salmonella* serovars.
250 Traditionally, feed and feed ingredients were thought to be an important contributor to being a
251 *Salmonella* spp. contact source for chickens during growth and under certain circumstances such
252 as breeder flocks this could have a considerable impact due to the vertical integration nature of
253 poultry production (Davies & Hinton, 2000; Jones & Richardson, 2004; Maciorowski et al.,
254 2004, 2006b, 2007; Ricke, 2005; Jarquin et al., 2009; Davies & Wales, 2010; Ge et al., 2013).
255 Therefore, it is not surprising that considerable effort has been made to not only implement

256 control measures in feeds and feed ingredients (Leeson & Marcotte, 1993; Ricke, 2003b, 2005;
257 Maciorowski et al., 2004, 2007; Wales et al., 2010; Jones, 2011), but to develop sensitive
258 detection methods for low levels of *Salmonella* potentially present in the feed matrix (Ricke et
259 al., 1998; Maciorowski et al., 2000, 2005, 2006a; Jarquin et al., 2009; Park et al., 2011, 2014;
260 Soria et al., 2011; Ricke et al., 2013c). Couple this with the fact that exposure to *S. Enteritidis*
261 can originate as a function of laying hens coming in contact with a wide range of carriers well
262 known for carrying *S. Enteritidis* such as insects, mice, rats, and other lesser known carriers in
263 the houses where these hens reside and it becomes clear that there are numerous and somewhat
264 unpredictable routes of exposure (Henzler, & Opitz, 1992; Hinkle & Hickle, 1999; Olsen &
265 Hammack, 2000; Davies & Breslin, 2001; De Reu et al., 2008; Lapuz et al., 2008; Park et al.,
266 2008; Holt et al., 2011; Galiş et al. 2013; Ricke et al., 2013a).

267 Before discussion on egg processing can begin, it is important to understand the
268 relationship between the serovar *S. Enteritidis* and why it particularly occurs as a contaminant
269 during all aspects of egg production. Historically, it became clear fairly early on that *S.*
270 *Enteritidis* possessed unique aspects that led to its being particularly problematic during egg
271 formation in laying hens (Guard-Petter, 2001; Holt, 1999, 2003, Ricke 2003a). One of the more
272 revealing pieces of evidence for this was the discovery that certain laying hen management
273 practices during egg production could enhance the likelihood of infection by *S. Enteritidis*. To
274 achieve additional egg laying cycles for commercial and economical reasons, laying hen egg
275 production can be halted, commonly referred to as molting, to literally shut down the
276 reproductive tract and in turn arrest egg production for a period of time prior to initiating a
277 second egg laying cycle (North & Bell, 1990; Bell, 2003; Berry, 2003; Park et al., 2004c; Ricke
278 et al., 2010, 2013a). The induction of a molt period can be done via altering the diet and is

279 physiologically manifested in a laying hen in a variety a ways including hormonally,
280 metabolically and immunologically among other detectable host responses (Berry, 2003;
281 Kuenzel, 2003; Park et al., 2004c; Dunkley et al., 2007a,b; Kim et al., 2012; Ricke et al., 2010,
282 2013a).

283 Probably the most effective means for inducing a molt in actively egg producing laying
284 hens was to simply to remove their feed for a period of time, then after sufficient time had lapsed
285 re-introduce these hens to feed once again (Bell, 2003; Berry, 2003; Yousaf & Chaudhry, 2008).
286 However, this led to the discovery that feed removal and subsequent emptying of the hen's
287 gastrointestinal tract created a localized microenvironment that was conducive to *S. Enteritidis*
288 colonization and systemic invasion (Durant et al., 1999; Holt, 1999, 2003; Ricke, 2003a;
289 Dunkley et al., 2009). This microenvironment change could be linked to enhanced expression of
290 pathogenesis genes in *S. Enteritidis* that corresponded to increased invasion and diminished
291 gastrointestinal microflora activity and in some cases microbial population levels as well (Durant
292 et al., 1999; Ricke 2003a; Dunkley et al., 2007c,d; 2009). While this was being established a
293 flurry of alternative dietary options were proposed and examined that avoided feed removal and
294 instead represented some sort of nutritional modification that would simultaneously ensure a
295 molt response in the hen while retaining relatively normal gastrointestinal tract
296 microenvironment and simulataneously serving as a barrier to *S. Enteritidis* (Corrier et al., 1997;
297 Seo et al., 2001; Berry, 2003; Holt, 2003; Ricke 2003a: Moore et al., 2004; Park et al., 2004a,b,c;
298 Woodward et al., 2005; McReynolds et al., 2006; Dunkley et al., 2007c,d; Donalson et al., 2008;
299 Ricke et al., 2010, 2013a).

300 Other means to limit *S. Enteritidis* establishment in laying hens have also been explored.
301 Administration of probiotic cultures that would lead to early establishment of a beneficial

302 gastrointestinal microflora that would in turn limit *S. Enteritidis* colonization have been explored
303 usually in young broiler chicks for *S. Typhimurium* control but some have been shown to be
304 effective against *S. Enteritidis* as well (Corrier et al., 1994a,b,1995; Nisbet et al., 1993, 1994,
305 1996a,b Ricke & Pillai, 1999; Nisbet, 2002; La Ragione & Woodward, 2003; Patterson &
306 Burkholder, 2003; Revollo et al., 2006; Higgins et al., 2007, 2008; Vilà et al., 2009; Hume,
307 2011; Siragusa & Ricke, 2012). Bacteriophages which can specifically lyse *S. Enteritidis*
308 bacteriophage have also been examined as a means to remove already established populations of
309 this pathogen either in table or fertile eggs or in the gastrointestinal tract (Borie et al., 2008;
310 Robeson et al., 2011; Bardina et al., 2012; Ricke et al., 2012a; Henriques et al., 2013; Spricigo et
311 al., 2013). Finally, there has been more interest generated on the development of live attenuated
312 vaccines that are based on genetic modifications of *S. Enteritidis* to render it less virulent but still
313 capable of inducing a hen to elicit a protective immune response against future exposure to *S.*
314 *Enteritidis* (Babu et al., 2003; Dewaele et al., 2012; Revollo & Ferreira, 2012).

315 In short, several extensive efforts have been made to short circuit establishment of *S.*
316 *Enteritidis* in laying hen production systems and many of these approaches have demonstrated
317 some success in decreasing the incidence of *S. Enteritidis* prior to egg collection and processing.
318 However, numerous opportunities for contamination by *S. Enteritidis* and other *Salmonella*
319 serovars can still occur during egg collection, processing, packaging, and in the final steps
320 leading to retail and home use. The remainder of this review will focus on the potential for
321 *Salmonella* contamination in the postharvest phases of egg production and processing.

322 **4.0 Egg processing and microbial contamination – General Aspects**

323 Table shell eggs continue to be a primary food source for human consumption either
324 directly as an intact shell cooked product, contents removed from egg shell components,

325 prepared in some fashion and cooked, or as an ingredient in a wide range of prepared foods and
326 baked goods. In 2009 alone, Americans consumed 250 eggs per person with total table eggs
327 being produced standing at 78.5 billion with 24 billion broken for use in egg products (Ricke et
328 al., 2013b). As discussed previously the U.S. egg industry has evolved tremendously in the past
329 century. As egg processing equipment became more technologically developed the number of
330 employees required to handle and inspect eggs decreased dramatically and the number of eggs
331 that could be processed and shipped for retail increased with the development of equipment
332 capable of washing, candling, sizing, and packaging over 180,000 eggs per hour (Musgrove,
333 2011). Eggs produced at the farm can enter the egg processing system either in an “in-line”
334 production system where eggs are directly moved via conveyor belts from the layer farm where
335 they are produced directly to an egg processing facility or as an “off-line” production system
336 where eggs are collected at the farm and subsequently transported to another site for processing
337 (Musgrove, 2011).

338 The multiple steps involved in egg processing represent numerous opportunities for
339 microbiological contamination of the egg as it progresses through the system from the hen to the
340 retail market. Identification of critical control points and conducting risk assessment studies have
341 been attempted over the years to assign and prioritize where the optimal strategies for limiting
342 exposure to *Salmonella* can be achieved during egg processing. Intuitively, some aspects such as
343 the presence of cracks in egg shells represent a risk for bacterial penetration and contamination
344 (Todd, 1996). Consequently, efforts to minimize crack formation and egg shell damage during
345 processing, packaging and transportation would be considered desirable. Alteration of the egg
346 shell surface integrity can easily occur to make it more vulnerable. For example, certain egg
347 sanitizers can actually cause physical damage to the egg shell surface leading to the increased

348 penetration of egg shell by surface microbial contaminants (Kim & Slavik, 1996; Wang &
349 Slavik, 1998).

350 Egg temperature has also been identified as a critical factor. Hutchinson et al (2004)
351 concluded that egg wash and rinse water temperature below 34 °C led to detectable egg content
352 contamination by *Salmonella*. However, this does not minimize risk from eggs that have become
353 internally contaminated by transovarian infection of *S. Enteritidis* while the egg is being formed
354 in the reproductive tract of the hen (Humphrey, 1994, 1999; Guard-Petter, 2001; Gantois et al.,
355 2009). Clearly with these contaminated eggs, strategies that minimize growth of *S. Enteritidis* to
356 keep populations low and developing methods to destroy this pathogen in the intact egg would
357 need to be considered. Combining rapid cooling and whole shell egg pasteurization have been
358 identified as effective means of reducing such risks (Whiting & Buchanan, 1997; Schroeder et
359 al.,2006). When summarizing more recent risk assessment studies, Chemaly & Salvat, (2011)
360 reported that rapid cooling would be helpful to reduce risk but that maintaining a continuous cold
361 chain to limit condensation was critical and under some circumstances rapid chilling could lead
362 to the formation of cracks in the egg shell. However, studies to evaluate all of the factors that
363 influence internal temperatures in eggs are limited and information on all of these factors
364 somewhat difficult to obtain.

365 A comprehensive study across several U.S. states was conducted to follow and compare
366 egg surface and internal temperature from oviposition to distribution to delineate some of these
367 factors for addressing risk questions raised by U.S. regulatory agencies regarding egg
368 temperature and time relationships over the course of egg production, processing and retail
369 distribution (Anderson et al., 2008; Koelkebeck et al., 2008; Patterson et al., 2008). Patterson et al.
370 (2008) compared external and internal egg temperatures during production at the layer house and

371 concluded that the two separate measurements correlated very highly with each other and were
372 influenced by state and seasonal differences. A similar seasonal and geographical impact was
373 noted for egg processing operations and as expected by the authors, temperatures were higher for
374 in-line operations compared to off-line operations since eggs from in-line systems come
375 immediately from the production house to be processed and would be inherently warmer
376 (Koelkebeck et al., 2008). Anderson et al (2008) when following temperature fluctuations of
377 refrigerated delivery trucks also noted a seasonal by delivery interaction impact on egg
378 temperature during transport for resale or distribution to retailers with less cooling occurring
379 during short delivery times.

380 In summary, some obvious control points such as occurrence of cracked eggs and
381 fluctuations in egg temperature have been identified as potential contributors to microbiological
382 problems associated with eggs during the production and processing cycle. However, despite the
383 efforts to provide more detailed information on certain aspects of egg production and processing,
384 many issues remain and *Salmonella* outbreaks originating from table shell eggs continue. As
385 discussed previously factors that contribute to layer hen infection and the corresponding vertical
386 and horizontal spread of *Salmonella* have been well documented by numerous authors (Guard-
387 Petter et al., 2001; Holt, 2003; 1999; Ricke, 2003a; Gantois et al., 2009; Ricke et al., 2013a).
388 Along these lines, interventions that are either currently implemented or proposed have been well
389 documented by many of these same authors. Consequently, these will not be discussed in this
390 review. Instead the focus in this review will be on *Salmonella* incidence along with general
391 microbial ecology and potential intervention measures during egg collection, processing, and
392 distribution. These aspects of the egg industry have not necessarily been ignored historically but
393 need to be revisited as the industry shifts more into alternative production systems and as the

394 international trade in eggs accelerates. Likewise, as consumers create market demands for small
395 scale locally produced eggs in developed countries and developing countries start to collect,
396 transport and distribute eggs from their small growers to more distant markets the opportunities
397 for contact with *Salmonella* needs to be reconsidered under the environmental conditions
398 imposed by these processing and retail systems. These issues will be addressed in the following
399 sections.

400 **5.0 Microbial contamination during egg collection at the farm to in-line processing**

401 The level of contact of eggs during the egg laying cycle with carriers and vectors of
402 *Salmonella* represent potential sources of contamination and have been well documented (Baron
403 & Jan, 2011). This would be of particular concern with chickens that have outdoors access such
404 as what occurs in some organic operations and certainly with free range chickens. Consequently,
405 limiting exposure to insects, mice, and other mobile carriers is warranted. Likewise, the
406 environmental exposure to layer hen fecal material during egg laying could be problematic.
407 Henzler et al. (1998) identified flocks with the highest level of *S. Enteritidis* contaminated
408 manure as being the greatest public health risk along with age of flock where birds that were
409 farther into the production cycle were of greater risk. This is supported by Hannah et al. (2011)
410 when they compared aerobic bacterial populations from washed and unwashed shell eggs from
411 caged and cage-free laying hens. Hannah et al. (2011) concluded that microbial levels were
412 generally lower in eggs collected from laying hens housed in cages with manure removal belts.

413 Henzler et al. (1998) also noted that eggs from contaminated egg handling equipment
414 were more likely to represent a greater risk. Contaminated egg equipment could be a key source
415 of cross contamination. Murase et al., (2001) used pulse-field gel electrophoresis patterns to
416 identify *Salmonella* serovars isolated from environmental samples of individual poultry houses

417 that were attached to an egg processing facility. They concluded that not only were the isolates
418 the same between the poultry houses and the egg processing facility but that the egg belts were
419 most likely to be a contributor to the dissemination of *Salmonella* serovars among houses. They
420 also noted that the same isolate could be continually recovered from a house for over a year. This
421 long term persistence has consequences not only as an ongoing source of contamination, but
422 indicates that cleaning and disinfection protocols may be somewhat ineffective and need to be
423 altered.

424 The type and composition of the egg belts may also be a factor. In an attempt to identify
425 environmental areas in Canadian egg production that could be difficult for cleaning and
426 sanitation programs Stocki et al. (2007) focused on egg conveyor belts. They pointed out that
427 egg conveyor belts used in battery cage operations could be a potential attachment surface for
428 persistence of *Salmonella* that also may be somewhat inaccessible to cleaning. In their study
429 when they compared egg conveyor belts composed of either belt material made of vinyl, nylon,
430 hemp, or plastic they observed that the vinyl conveyor belt material which had the least surface
431 area for *Salmonella* colonization also resulted in the lowest recovery of *S. Typhimurium* and *S.*
432 *Enteritidis* after washing and disinfection while hemp-plastic belt material had the greatest
433 surface area and the corresponding highest level of remaining *S. Typhimurium* and *S. Enteritidis*.
434 They concluded that conveyor belts made of woven materials were more susceptible to persistent
435 and recalcitrant *Salmonella* colonization, and conversely belt materials with less surface area
436 such as vinyl were better for effective *Salmonella* removal.

437 **6.0 Microbial contamination during transportation to off-line egg processing facilities**

438 Some of the general issues discussed with egg collection and microbial contamination
439 would be similar for off-line processing but other unique factors have been identified for these

440 operations as well. Knape et (2002) compared microbial contamination levels in specific time
441 intervals between on-line and off-line processing systems at four sites, namely the egg collection
442 conveyor belt, during detergent application prior to exposure to the sanitizer, immediately after
443 administering the sanitizer, and immediately before packaging. Using aerobic microbial plate
444 counts as their microbial contamination monitor they noted that for the most part microbial
445 populations were greater as the processing shift advanced over time in the off-line egg
446 processing operations compared to the in-line operations. In particular by the time eggs were
447 ready for packaging off-line aerobic bacterial contamination was greater at all time points versus
448 in-line microbial contamination. They concluded that this may in part be due to the eggs arriving
449 at the processing site were fresher for on-line production systems thus allowing less time for
450 attachment and growth of microbial contaminants as well as less time for the organic material to
451 adhere that might further facilitate microbial contamination and interfere with cleaning of the
452 eggs.

453 Knape et al. (2002) also speculated that storage time may contribute to the higher levels
454 of microbial contamination in off-line egg processing operations by enhancing adherence of
455 organic matter on the shell as well as allowing for bacterial penetration of the egg shell.
456 Musgrove and coworkers examined this in more detail by focusing on the nest run carts that
457 consist of plastic flats placed on metal carts with unpainted wooden shelves used to transport
458 eggs from the layer houses to the off-line egg processing facilities. As pointed out by Musgrove
459 et al (2012) such carts may become problematic as eggs can become cracked, subsequently
460 leaking onto the wooden shelves which in turn are used indefinitely and typically are not part of
461 the sanitation regime. This was based on earlier work where Musgrove et al. (2004) surveyed 14
462 non-egg-contact surface multi-state commercial egg in-line, off-line and mixed (both in-line and

463 off-line) processors for general aerobic and *Enterobacteriaceae* bacterial populations at the end of
464 the processing day and before the next processing shift to assess sanitation impact. Of the
465 various sites sampled, nest cart shelves and nest cart wheels yielded some of the highest
466 microbial population counts with statistically non-significant differences between processing
467 times.

468 In a followup study, Musgrove et al (2009) identified the isolates of *Enterobacteriaceae*
469 from nest cart shelves to belong to the genera *Citrobacter* spp., *Escherichia* spp., *Enterobacter*
470 spp., *Klebsiella* spp., *Hafnia* spp., *Kluyvera* spp., *Leclercia* spp., and *Salmonella* spp.,
471 respectively and concluded that the shelves could serve as a reservoir for Enterobacteriaceae
472 including foodborne pathogens such as *Salmonella*. They confirmed this when they sampled
473 shelves from nest carts used in off-line and mixed line operations and preenriched for *Salmonella*
474 isolation and further identification (Musgrove et al., 2012). Shelves from off-line operations
475 were 3-fold (36% total positives) more contaminated with *Salmonella* spp. with serotypes *S.*
476 *Cerro*, *S. Heidelberg*, *S. Infantis*, *S. Mbandanka*, and *S. Oranienberg* recovered from the off-line
477 operation and serotypes *S. Anatum*, *S. Mbandanka*, and *S. Typhimurium* recovered from the
478 mixed operation facility.

479 **8.0 Microbial contamination during egg processing**

480 Once eggs are received in the egg processing plant either from on-line or off-line
481 operations, additional opportunities for microbial cross contamination can occur during egg
482 processing and subsequent packaging for retail shipment. Typically eggs arrive on conveyor
483 belts from the corresponding on-line and off-line production sources go through an accumulator,
484 followed by an egg washer, subsequently dried, oiled, candled and electronically checked for
485 cracks, weighed, sorted, and finally packed for retail distribution (Musgrove, 2011). Much of the

486 egg handling during processing utilizes vacuum loaders, electronic surveillance and weighing
487 systems enabling many more eggs to be processed and prepared for retail (Musgrove, 2011).
488 However, despite the trend in increased automation, several research studies indicate that
489 microbial and *Salmonella* cross contamination during egg processing still remain part of the
490 ongoing concern as *Salmonella* outbreaks continue to occur (Davies & Breslin, 2003).

491 Early work by Jones et al. (1995) assessed *Salmonella* frequency from environmental
492 samples, and external and internal egg contents collected at egg layer farms and an on-line
493 processing facility in the U.S. and concluded that although approximately 1 % of the
494 commercial eggs were positive for *Salmonella* serovars externally, internal contamination was
495 relatively infrequent. In a comprehensive U.K. field-based farm-egg packing plant study, Davies
496 & Breslin (2003) sampled the packing plant floor, grading table, candlers, conveyor belts, rollers
497 and handler tables either during operation or prior to the respective plant's disinfection schedule.
498 They observed that all sites sampled were overall 20 to 30% contaminated with *Salmonella* and
499 that these levels reflected the level of contamination with the corresponding layer house with
500 eggs undergoing longer distances on roller conveyors prior to reaching the packing plant being
501 less contaminated. To examine potential for cross contamination of eggs during processing in
502 these packing plants, they sterilized eggs by boiling for 10 minutes and subsequently sent them
503 through the packing plant 1 to 3 times, followed by culturing them for *Salmonella*. Although
504 they did not detect *Salmonella* from sterile eggs passed through the plant once, by the third time
505 they observed a minimum acquisition contamination rate of 0.3%. They concluded that
506 disinfection only reduced these levels somewhat, which they believed was a reflection of their
507 observation of generally poor cleaning in the egg packing plants resulting in detectable residual
508 contamination.

509 Jones et al (2003) assessed the effectiveness of sanitation programs in southeastern U.S.
510 egg processing plants by comparing on-line, off-line and mixed line shell egg processing plants
511 located in different states either at the end of the operating day or the next morning prior to the
512 start of operations. They used sterile gauze pads to sample sixteen direct and indirect contact
513 sites within these plants including belts, scales, check detector, vacuum loaders, egg washer tank
514 brushes, washer lids and tank walls, oiler flaps, and packer head belts, among others for
515 determining the levels of aerobic bacteria and *Enterobacteriaceae*. In general, they could not
516 detect aerobic bacterial population differences between end of the operating day versus the next
517 morning which has also been reported in other studies (Musgrove et al., 2004). However, they
518 did observe high levels of bacteria recovered from the washer tank wall, the rewash belt and the
519 vacuum loaders with bacterial counts becoming lower as the eggs moved further along into
520 cleaner areas of the processing line. They concluded that there is the potential need for
521 development and application of cleaning chemicals that better take into account the challenges of
522 a commercial operation and that sanitation could potentially be made more effective if separated
523 into cleaning zones based on the particular types of equipment involved in the different
524 components of the egg packing plant operation.

525 Musgrove et al. (2005) conducted an intensive microbial examination of the individual
526 stages of egg processing in mixed line operations by using egg shell rinses of eggs collected at
527 different processing sites and enumerating total aerobic bacteria, yeast and molds, *E. coli*, and
528 *Enterobacteriaceae* along with preenrichment for *Salmonella* isolation. They noted that generally
529 microbial populations from shell rinses were highest at the accumulator and the rewash belt.
530 They also concluded that prevalence for individual groups of bacteria for the most part also
531 generally declined as eggs transitioned from preprocessing to post-processing while overall

532 *Salmonella* prevalence ranged at approximately 10% with considerable differences between
533 individual processing plants. They also observed that *Salmonella* was isolated at every site in all
534 stages of egg processing at least once when combining all isolations across all visits and plants.
535 In a followup study, Musgrove et al. (2008) used commercial biochemical-based identification
536 assays and identified *Escherichia coli* and *Enterobacter* spp. from most of the plants with lesser
537 frequencies of other *Enterobacteriaceae* genera including *Citrobacter* and *Klebsiella* and non-
538 *Enterobacteriaceae* bacteria including *Pseudomonas*, *Aeromonas* and *Vibrio* among others.

539 When Jones & Musgrove (2008a,b) examined sterile saline rinsates from vacuum loaders
540 which are used to transfer eggs from incoming egg flats in mixed and off-site egg production
541 facilities, they observed higher aerobic bacterial populations in mixed operations but higher
542 *Enterobacteriaceae* in the off-line facility. Based on biochemical test, predominant bacteria
543 included *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter*, and *Serratia*. *Campylobacter* and
544 *Salmonella* were recovered at rates of 1.6 and 3.3 %, respectively, while *Listeria* were recovered
545 in 72 % of the samples (of which 98.8% were *L. innocua* and 1.2% were *L. monocytogenes*). The
546 authors speculated that vacuum loader cups could serve as a reservoir for *Listeria*. They also
547 concluded that bacterial growth niches may be appearing in these loader cups thus stressing the
548 need for better cleaning and sanitation approaches for these particular devices. It is not clear
549 where the *Listeria* may be originating from as they seem to be linked with facilities that have off-
550 line capabilities. Whether they originally were attached to incoming egg flat surfaces or were
551 part of the plant facility environment microflora remains to be determined. Certainly, *Listeria*
552 spp. are well known inhabitants of a wide range of food processing plant environments (Milillo
553 et al. 2012a) but several species have been isolated from poultry origins as well including pasture
554 flock poultry birds, eggs and egg wash water (Laird et al, 1991; Farber et al., 1992; Jones et al,

555 2006; Milillo et al., 2012b). More surveillance research needs to be done to determine the extent
556 of *Listeria* contamination in egg processing plants and the potential risk to public health.

557 **9.0 Egg wash water and sanitation**

558 Several countries such as the United States, Japan, and Canada wash and grade eggs prior
559 to shipping them out for retail distribution, but egg washing is generally not allowed or practiced
560 in the European Union (Hutchinson, 2003; Musgrove, 2011; Howard et al., 2012). As described
561 by Hutchinson (2003) the egg washing process essentially involves four steps, namely wetting,
562 washing, rinsing and drying. Egg washing procedures conducted in commercial operations
563 typically employ a mechanical washing apparatus that mists an alkaline detergent solution across
564 eggs as they are brushed from side to side while moving along the egg conveyor belt followed by
565 a final rinse usually containing some sort of chlorine or quaternary based sanitizer (Hutchinson et
566 al., 2003; Northcutt et al., 2005; Howard et al., 2012). Once the eggs have been washed the
567 remaining egg wash and rinse waters are combined, filtered and recirculated to a wash tank
568 where they can be reheated for repeat usage in the initial wetting and cleaning stages of the egg
569 washer cycle (Hutchinson, 2003; Northcutt et al., 2005; Howard et al. 2012). Details on the
570 development, management and application of egg wash water has been extensively reviewed
571 elsewhere and will only be briefly discussed in the current review (Moats, 1981; Hutchinson et
572 al., 2003; Messens, 2011).

573 While reusing egg wash water to minimize overall water use is desirable there are several
574 potential issues with this reuse of egg wash water (Hutchinson, 2003). Even though the alkaline
575 pH of the egg wash water is designed to not only clean egg shells but limit bacterial proliferation
576 accumulation of organic matter from recirculation can dilute its efficacy (Kinner and Moats,
577 1981; Howard et al., 2012). To address this, Northcutt et al. (2005) profiled the potential

578 fluctuations in the composition of commercial shell egg wash water from three in-line egg
579 processors in southeastern U.S. by monitoring temperature, pH, chlorine, soluble iron (ferrous),
580 total dissolved solids, total suspended solids, total Kjeldahl nitrogen, and chemical oxygen
581 demand. They compared fresh incoming tap water with water sampled after the first 2 hours of
582 operation from two egg shell washers that used an end-to-end arrangement as a dual washer
583 system in these facilities. In general they observed considerable variation among plants for most
584 of these parameters and concluded that differences occurred in age of plant and equipment,
585 management of the plant, source of eggs, number of broken eggs among other factors. In
586 particular when decreases in pH and increases in soluble iron occur, these could be especially
587 problematic as both can lead to increases in bacterial growth and contamination including
588 *Salmonella* spp. if they reach certain levels where they no longer limit bacterial growth and
589 proliferation (Hutchinson et al., 2003; Messens, 2011; Howard et al., 2012).

590 The fluctuations in chemical composition raise the general issue as to whether egg
591 washing effectively limits microbial contamination. To determine this, Jones et al. (2004)
592 profiled microbial populations by collecting eggs before and after egg processing over three
593 consecutive days from a commercial in-line processor with a dual tank egg washer. They stored
594 eggs over ten weeks of storage and enumerated total aerobic bacteria, pseudomonads, yeast,
595 molds and *Enterobacteriaceae* from both external egg shell surface rinses and internal egg
596 contents. While they did not observe differences in total aerobic bacteria, yeasts and molds
597 between unwashed and washed egg contents, they did report substantially less external total
598 aerobic bacterial, and yeast and mold populations in washed eggs compared to unwashed eggs
599 while only low levels of *Enterobacteriaceae* and pseudomonads were detected in any of the eggs
600 sampled. It would be of interest to develop more detailed microbial profiles of egg shell

601 microbial populations to determine shifts in specific groups and even individual microorganisms
602 during egg processing. Likewise, the interaction between microflora on incoming eggs versus
603 resident egg processing plant microbial populations would be helpful to elucidate their respective
604 impacts on the efficacy of egg washes and sanitizers. As microbial identification technology
605 progresses with next generation sequence capabilities becoming more routine and readily
606 available, extensive characterization of these corresponding egg processing plant and egg shell
607 surface microbiomes may become a reality.

608 Despite the apparent effectiveness of egg washing, as Jones et al. (2004) pointed out from
609 their earlier studies (Jones et al., 2003), reduced bacterial contamination is not always observed
610 in commercial egg processing operations. This occurs despite the employment of some sort of
611 chemical sanitizer during the egg processing cycle. Sanitizers used in egg processor facilities are
612 generally applied immediately after the alkaline egg wash cleaning step as a rinse solution
613 containing a chlorine concentration of 100 to 200 ppm, or a quaternary ammonium-based
614 compound (Hutchinson et al., 2003; Howard et al., 2012). Given the inconsistencies in effective
615 microbial contamination control and issues such as reducing cost, a wide range of sanitizers and
616 other decontamination technologies for potential use in egg processing have been examined over
617 the past several years (Howard et al., 2012). Individual compounds and other strategies have
618 been described extensively elsewhere (Berardinelli et al., 2011; Howard et al., 2012; Galiş et al.
619 2013) and will only be briefly discussed here. Generally, the sanitizer approaches can be
620 classified as application of either unique chemical or biological compounds administered in a
621 similar manner as the more traditional sanitizer compounds. These would include agents such as
622 botanical compounds, enzyme catalyzed bactericidal reactions, or electrolyzed water (Kuo et al.,
623 1997b; McKee et al., 1998; Knape et al., 1999, 2001; Russell, 2003; Bialka et al., 2004; Park et

624 al., 2005; Cao et al., 2009; Upadhyaya et al., 2013). Nonchemical approaches to
625 decontamination include subjecting shell eggs or egg processing equipment to some sort of light,
626 gas or other form of radiation that is bactericidal such as ultraviolet light, non-thermal
627 atmospheric gas plasma, ozone, or ionizing radiation (Gao et al., 1997; Kuo et al., 1997a,c; ;
628 Chavez et al., 2002; Rodriguez-Romo & Yousef, 2005; Keklik et al.,2010, Ragni et al., 2010).
629 Although many of these approaches show promise much remains to be done to assess
630 effectiveness under different processing conditions as well as the need to address cost and
631 economics for scaling up some of these technologies.

632 **10.0 Egg retail and microbial contamination**

633 Once eggs are processed and sorted they are packaged for retail and distributed to retail
634 markets. Starting in the 1970's considerable effort was made to compare different types of egg
635 packaging that was best suited not only for retail market display but protected eggs from damage
636 to the shell during transportation as they traveled to their respective market destinations. Most of
637 the research and development focus was directed toward either expanded polystyrene (EPS) or
638 molded paper pulp (MPP). Early work addressed issues such as ability of particular egg carton
639 types to cushion eggs sufficiently to minimize crack formation (Mellor and Gardner, 1970, 1975;
640 Mellor et al., 1975; Denton et al., 1981; Lederer, 1983; Nethercote et al. 1974; Seydim &
641 Dawson, 1999). Nethercote et al. (1974) concluded that for both polystyrene and molded pulp
642 cartons that design was more important than carton material. Much of the later work has been
643 directed towards the response of these types of egg cartons to temperature fluctuations during
644 storage and transport and rapid cooling systems (Thompson et al., 2000).

645 While egg packaging has evolved to a large extent in the U.S., potential contamination
646 issues remain with certain segments of the egg retail market. Historically, used cases, fillers and

647 flats were considered readily available for reuse and this apparently occurred where deficits were
648 present (Eggleton & Carpenter, 1961). However, Eggleton & Carpenter (1961) after surveying
649 the egg industry at that time recommended that damaged and soiled flats needed to be avoided
650 and controls should be exercised over reuse policies. Board et al. (1963) surveyed new, used, and
651 dirty egg flats and observed that they could become heavily contaminated especially if they had
652 egg albumen or yolk material remaining on them. Banwart, (1964) demonstrated that *Salmonella*
653 and other egg contaminant bacteria could attach to these egg flats and that only autoclaving the
654 flats completely eliminated them.

655 This issue has re-emerged for certain local markets where retail egg containers can be
656 reused and there is the potential for contamination to occur over time if these are not properly
657 sanitized. There is evidence for this potential risk based on studies conducted on retail egg
658 markets in other countries. Suresh et al., (2006) collected samples of egg shells, egg contents,
659 and egg trays in South India retail markets and isolated and identified *Salmonella* spp. from these
660 sources. Based on the recovered levels of *Salmonella* from these various sources they concluded
661 that reused egg trays were a potential risk for exposure to *Salmonella*. In a later study, Singh et
662 al., (2010) profiled eggs transported from farms to wholesale and retail markets located in North
663 India in an attempt to characterize and track where *Salmonella* cross contamination might be
664 occurring. *S. Typhimurium* was the predominant serovar and they observed a higher incidence
665 from eggs collected in the retail markets versus the levels they isolated in the fresh eggs from the
666 egg layer farms. Based on these comparisons, they suggested that surface contamination must
667 have occurred during handling, storage, and transportation of the eggs from the farms to the
668 market. Utrarachkij et al., (2012) conducted an in-depth study on the level of *Salmonella*
669 contamination in samples they had collected from eggshells, egg contents, reusable egg trays and

670 associated environments in Thailand egg farms and markets. After a comparison among these
671 multiple sources they concluded that reusable egg trays used for these eggs could serve as a
672 potential source of horizontal *Salmonella* transmission.

673 Based on these studies it is clear that little is known about the interaction between the type
674 of retail packaging and the cross contamination that may occur between it and the table shell egg.
675 Certainly opportunities exist for potential microbial cross contamination depending on the type
676 of packaging material, particularly if it is reused and not properly cleaned. More comprehensive
677 microbial studies need to be conducted on different types of packaging materials to better
678 identify the dynamics of microbial diversity and their potential interactions with foodborne
679 pathogens such as *Salmonella* spp. As discussed with egg processing, advanced molecular
680 technologies such as complete microbiome sequencing offer opportunities to much more
681 thoroughly characterize these microbial population and delineate patterns that may contribute to
682 persistent contamination problems. This is critical as some of these complex microbial
683 populations that can be present on some surfaces may possess capabilities of forming biofilms
684 which could harbor pathogens thus rendering the pathogens somewhat protected from typical
685 sanitation protocols and administration of sanitizing agents. The other development that may
686 prove useful to egg packaging is the development of unique packaging materials that possess
687 antimicrobial characteristics that could serve as a barrier for preventing cross contamination.
688 There is precedent for this in the food industry with other food commodities (Suppakul et al.
689 2003). One could envision such packaging not only containing antimicrobials but as
690 technologies develop possessing sensor capabilities for monitoring environmental conditions
691 such as temperatures along with precise tracking capabilities and perhaps eventually
692 incorporating direct microbial contamination level indicators.

693 **11.0 Conclusions and future directions**

694 Salmonellosis continues to be major cause of foodborne disease in the U.S. with over one
695 million cases occurring annually costing several billion dollars (Scallan et al., 2011; Foley, et al.,
696 2013). Poultry products are still one of the major sources of Salmonella in the U.S. (Foley et al.,
697 2011; 2013; Painter et al., 2013). *Salmonella* and microbial contamination continue to be issues
698 for the commercial egg industry, but the sources and cross contamination routes have changed
699 over time. In addition, different *Salmonella* serovars have become more closely identified with
700 eggs, such as *S. Enteritidis* which appears to have some host affinity for infecting laying hens.
701 This is partly due to the evolution of *Salmonella* serovars over the years and also to some extent
702 the result of changes in egg production management. In addition, the impact of recent
703 salmonellosis egg associated outbreaks have been more prominent from a public health
704 standpoint, not only because of the larger egg operations involved but also because of the more
705 comprehensive and advanced molecular-based trace back technologies that are now available
706 and can be routinely implemented. These trends will remain as the commercial U.S. egg
707 industry continues to evolve from a small flock local market in the last century to the large flock-
708 multiple house and egg processing plant complex production systems common to the current
709 industry. However, as the egg industry has developed into a vertically integrated system, more
710 narrowly focused commercial niche markets have emerged such as speciality or designer eggs
711 along with the ever increasing consumer demand for organic and free range layer flocks
712 produced eggs. Some of these systems such as free range chickens present additional challenges
713 for managing food safety. Likewise, as the egg industry shifts more internationally and trade
714 between countries expands, food safety issues associated with these eggs may also become more
715 prevalent.

716 However, much has been learned about the ecology of *Salmonella* in layer flocks and
717 how this influences subsequent egg contamination. While most *Salmonella* penetrate eggs from
718 the exterior shell, *S. Enteritidis* has shown the ability to infect hens and colonize the reproductive
719 tract resulting in contamination of the internal contents of the egg. Several environmental factors
720 influenced this, but certain management practices were identified as primary culprits for creating
721 a gastrointestinal tract microenvironment that enhanced *S. Enteritidis* invasiveness. Since these
722 factors became known, a number of management strategies have emerged to limit this. Likewise,
723 as eggs are collected from the farm and either sent directly via conveyor belt to an on-line egg
724 processing plant or transported to an off-line processing plant, more is now known about the
725 microbial ecology of eggs as they enter the plant. Problem areas still occur, in particular during
726 transport to off-line facilities and the use of nest carts. Other environmental areas for microbial
727 contamination include certain types of conveyor belts and vacuum loaders. During egg
728 processing the use of egg washes and sanitizers can help to reduce the bacterial load during egg
729 processing but improvements and more sanitizer options are needed. In particular, it appears that
730 *Salmonella* cross contamination of external egg surfaces can occur during processing. Finally,
731 opportunities exist for potential contamination at the retail market depending on the type of
732 packaging material. Clearly, reuse of egg trays as seen in certain parts of the world have been
733 identified as a potential high risk source of *Salmonella* contamination. This is supported by work
734 in U.S. during the early 1960's which demonstrated that trays soiled by egg contents that had
735 been reused were easily contaminated by *Salmonella* attachment to these surfaces and the
736 pathogen could only be eliminated by the extreme measure of autoclaving. This may be
737 particularly important in local egg retail markets where egg containers may be reused without

738 proper cleaning. Clearly, efforts to better communicate risks to the public may be warranted as
739 well.

740 There continues to be a need for improvement in food safety especially as it is associated
741 with all aspects of egg production, processing and retail. This will require not only further
742 development of control measures, but detailed identification of critical high risk areas where
743 microbial contamination is most likely to occur. Consequently, an in-depth profile of the
744 microbial populations involved at various stages of egg production, particularly during transport,
745 arrival at the egg processing plant and during the various stages of egg washing, oiling, sorting
746 and packaging is needed. Advanced molecular approaches such as microbiome analyses offer
747 intriguing possibilities to provide more details on how environmental factors such as changes in
748 egg wash water chemistry and temperature may influence the microflora on the egg shell surface.
749 Likewise, functional genomic analysis offers the opportunity to understand how *Salmonella* spp.
750 may respond to these same conditions. Identifying responses at the genomic level may lead to
751 development of unique antimicrobials and sanitizer strategies that can more precisely target
752 *Salmonella* spp. at all phases of egg production. The ultimate goal will be to produce a safer egg
753 with a longer shelf life at the retail market.

754

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