# *Listeria* in Conventional and Alternative Egg Production Systems

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*Listeria* continues to be a persistent foodborne pathogen that is responsible for human cases of listeriosis when contaminated food products are consumed. Human subjects considered to be most susceptible include the elderly, immunocompromised, and pregnant women. *Listeria* is characterized as a saprophytic organism with the capability of responding and adapting to constantly changing environments because it possesses multiple stress response mechanisms to overcome varying temperatures, salt concentrations, and pH, among others. Primary foods and food products associated with listeriosis include dairy products and ready-to-eat meats such as turkey products. Historically, chicken eggs have not been identified as a primary source of *Listeria,* but the potential for contamination during egg production and processing does exist. *Listeria* species have been isolated from egg-processing plant equipment and are presumed to occur in egg-processing plant environments. Whether *Listeria* is consistently disseminated onto eggs beyond the egg-processing plant is a risk factor that remains to be determined.

Keywords: Listeria ; poultry ; eggs

## 1. Introduction

*Listeria monocytogenes* is a foodborne pathogen of notable concern, especially due to its growth under household refrigeration temperatures (40 °F, 4 °C) and at a pH range of 4.5 to 9.6 [1][2][3]. *Listeria monocytogenes* has been subdivided into four phylogenetic evolutionary lineages due to variations in ecology recombination rates and genomic content [4][5]. Lineage I consist of most human clinical isolates and includes serotypes 1/2b, 3b, 3c, and 4b strain [5][6]. Lineage II is more prominent in environmental samples, foods, and animals and includes 1/2a, 1/2c, and 3a, while Lineages III and IV are mainly found in ruminants [7]. In human cases of listeriosis stemming from chicken meat, serotype 1/2b is the most prominent [8]. However, serotypes 1/2a and 1/2b are predominately isolated from ready-to-eat (RTE) foods [9][10], and Gilbreth et al. [11] noted that clinical isolates from patients made ill by RTE foods were mostly 1/2a or 4b. Severe infections of *L. monocytogenes* can occur in the elderly, immunocompromised, and pregnant women and can have a mortality rate exceeding 20% [12][13][14]. In 2021, the last year for which data are available, 2268 confirmed cases of listeriosis were reported by 30 EU/EEA Member States, a rate of 0.51 per 100,000 population [15]. In that same year in the U.S., there were approximately 1029 laboratory-confirmed domestically acquired cases, a rate of 0.31 per 100,000 [16]. The infective dose of *L. monocytogenes* varies with the strain of *L. monocytogenes* and the susceptibility of the victim, with pregnant women, newborns, the elderly, and immunocompromised persons being the most vulnerable [17]. Epidemiological modeling of the 2015 United States (U.S.) *Listeria* outbreak in ice cream indicated that in each gram of product, 620 colony forming units (CFU) *L. monocytogenes* cells were present and that the highly exposed population ingested $7.2 \times 10^6$ to $3.3 \times 10^7$ cells despite only four cases being reported [18]. Due to growth conditions and mortality rate, there is a "zero tolerance" policy for *Listeria* (1 CFU/25 g product) in ready-to-eat (RTE) foods in the U.S. [19].

## 2. Egg Production and Food Safety

Raw eggs are not considered RTE foods and, in many cases, are cooked thoroughly, which inactivates pathogens [20]. However, the consumption of raw cookie dough or mayonnaise (which contains eggs), as well as raw egg use in protein shakes, suggests that they could serve as a potential vector for listeriosis [21]. Federal regulatory agencies in the U.S. do not oversee farms that contain less than 3000 laying hens, and these farms are not required to register under the USDA's Egg Products Inspection Act (EPIA), which many pasture poultry farms may fall under [20][22][23].

The cartons of eggs consumers purchase have code dates printed on them that may be best by, best if used by, or an expiration date [24]. An expiration date can be no longer than 30 days from the date the eggs were packed into the carton, while sell-by or use-by dates can be no more than 45 days from the date the eggs were placed in the carton [24]. The USDA recommends that eggs be cooked to 160 F (71 °C) and that shell eggs should not be washed as bacteria such as *Salmonella* can be "sucked" into the egg through pores during washing [24]. USDA also recommends using pasteurized eggs or egg products when preparing recipes that call for using eggs raw or undercooked [24].

## 3. *Listeria* in Poultry Production

There are several studies regarding the contamination of broiler flocks and broiler processing plants with *L. monocytogenes.* These studies were extensively reviewed by Rothrock et al. [5][25] but are briefly discussed here. Cox et al. [26] found that 6% of eggshell fragments in a U.S. commercial broiler hatchery tested positive for *L. monocytogenes*. Despite this, some studies have reported a 5% and 14% prevalence of *Listeria* in fecal samples [27][28]. In the first round of rearing, before broilers were placed, pastured poultry grass and soil samples yielded 8 isolates of *Listeria,* while after broilers were added, 13 serotypes were isolated [29]. In the second round of rearing, no isolates were obtained from soil and grass before the introduction of broilers, but 19 isolates were found after broilers were placed [29]. In processing facilities, floor drains are an important niche for *Listeria* persistence. Blackman and Frank [30] and Berrang et al. [31] performed experiments to determine whether a spray of water into a contaminated drain could carry *Listeria* cells to broiler meat on a table 2.4 m away from the drain. In Brazil, 14.4% of samples collected from post-evisceration poultry products in a plant with automatic evisceration tested positive for *L. monocytogenes,* as opposed to a plant with manual evisceration, which had 19.4% positive [32]. On retail products, Miranda et al. [33] found that 49.1% of organic and 41% of conventionally grown broiler drumsticks were positive for *L. monocytogenes*. Elmali et al. [34] collected and cultured broiler wing meat purchased from supermarkets and determined that 57/120 samples were positive for *Listeria* spp. (47.5%), and 54 of the 57 samples were identified to be *L. monocytogenes*.

## 4. Layer Hens and Egg Production Systems

Commercial layer hens begin their life in the hatchery after a 21-day incubation and are placed on a farm within 12 to 48 h of hatching [35]. In cage-based production systems, these immature chickens, commonly referred to as pullets, are raised in cages separate from the mature birds for 18 weeks and are not given more than 10 h of daily light to prevent premature egg laying [35]. Birds are subsequently transferred to a layer house and placed in larger cages; they begin producing eggs around 20 to 21 weeks and efficiently lay for 52 to 60 weeks [36]. The birds then typically begin to go through a molting process to stop egg production, followed by rejuvenation for another egg-laying cycle [37]. After molting, layer hens usually produce larger but poorer-quality eggs for a shorter period [35]. Depending on economic factors, including flock size and feed costs, hens may undergo one to three laying cycles before they are replaced [35].

Farmers, particularly those of backyard flocks, may choose to hatch their eggs instead of receiving them from a hatchery. However, small chicks are at risk from predation from rats, as well as household pets such as dogs and cats [38]. The USDA defines free-range as any system that provides limited access to a fenced-in outdoor area [39][40]. Pasture poultry refers to using a mobile coop with nest boxes with constant daytime access to the outdoors and typical coop rotation to ensure pasture vitality [39][40]. However, this definition varies as the certified humane organization requires 2 $ft^2$ (0.2 $m^2$) of outdoor access to the outdoors and at least six hours of access to the outdoors to be considered free-range, and for pasture-raised birds, 108 $ft^2$ (10 $m^2$) per bird of grass is required with the fields being rotated [41].

## 5. *Listeria* Presence in Layer Hens and the Environment

Free-range and pasture systems can expose the hens to wild birds, rodents, and other vectors that can harbor *Listeria* [25][42][43]. As in humans, *L. monocytogenes* can cause septicemia and localized encephalitis in adult poultry; young chicks are more susceptible to a chronic form of infection, and the mortality rate in these young birds can be as high as 40% [44]. Adult chickens and turkeys are relatively resistant to experimental infection and are thought to be prime reservoirs for contamination of the litter and environment of poultry production houses [45][46]. The relative resistance was supported by a three-trial study, where it was demonstrated that oral infection of 6 logs *L. monocytogenes* CFU/mL on day 14 or day 35 broiler chickens did not result in *L. monocytogenes* recovery in the gastrointestinal tract for two of the trials on day 42, while day 1 infections resulted in the recovery of the pathogen [46]. There are no characteristic signs of listeriosis in poultry despite occasional meningoencephalitis and symptoms from septicemia [44].

Chemaly et al. [47] investigated the prevalence of *L. monocytogenes* in French layer flocks. Dust from the environments of 200 laying hen flocks was sampled, with 88 flocks reared in cages and 112 reared in floor pens. Of the caged hens, 7 of 139 dust samples were positive for *L. monocytogenes,* whereas only 6 of 206 dust samples from the floor-reared flocks were positive [47]. Aury et al. [48] also surveyed French layer hen flocks but focused only on caged flocks with more than 1000 hens. By sampling the fecal material, they were able to determine that of the 84 flocks, 25 (31%) tested positive for *Listeria*. These levels of prevalence are similar to the prevalence of *L. monocytogenes* found in the fecal material of broilers, including free-range flocks [5][47][48][49].

Domestic fowl have been suggested as potential vehicles to transmit *Listeria* to human beings [50]. Risk factors for caged hens being infected with *L. monocytogenes* were detailed by Aury et al. [48]. They concluded that the presence of pets on the production sites, such as on many backyard farms, increased the risk of *L. monocytogenes* in layers flocks. Weber et al. [51] indicated that *L. monocytogenes* could exist in pets, and they can play a role as a vector. Layer feed can also play a role in the transmission of *L. monocytogenes,* and this pathogen has been detected in pelleted feed [52][53]. Caged layer hens had a decreased risk of acquiring *L. monocytogenes* if deep pit storage and conveyor belt system was used to dispense with fecal material (11.8% positive) as compared to deep pit alone (16.7%) or conveyor belt with dunghill

storage (42.9%) [48]. The poultry red mite (PRM), *Dermanyssus gallinae*, is a ubiquitous parasite infesting egg-laying hens and can be a vector for pathogens [54].

Garcia et al. [55] evaluated the impact of the use of cattle manure on the presence of *Listeria* in the environment and eggs in free-range flocks. No statistically significant differences in levels of *Listeria* in the environment and eggs were found between those raised without manure (15.7% positive) and those raised with manure in the pasture (17.1%) [55]. The density of stocking has also been related to contamination with *Listeria*; layers were maintained at low (4.02 $m^2$/bird) or high (2.01 $m^2$/bird), and the eggs and the environment were assessed for the presence of *Listeria* [56]. *Listeria* was detected on the shells of 7.5% of the eggs and in the contents of 2.5% of the eggs in the low-density population, as opposed to 17.5% on the shells and 7.5% of the contents in the high-density group, respectively [56].

Crespo et al. [57] reported an unusual case of listeriosis in chickens in a backyard flock in Washington state. The flock included 20 egg-laying chickens approximately eight months old; over a five-month period, seven birds had died, and five birds exhibited depression, anorexia, and panting [57]. Two of the dead birds were presented for necropsy, and *L. monocytogenes* 4b was cultivated from multiple organs; the source of the infection was not discovered [57]. The family continued to consume eggs from the flock, and no illnesses were reported among them. Many species of birds are susceptible to infection by *L. monocytogenes*, although clinical disease in birds is rare. It is important to note the possibility of transmission of this pathogen to humans via direct contact or preparing and consuming chicken meat or eggs from contaminated flocks.

## 6. *Listeria's* Presence in Egg-Processing Facilities

Egg-processing facilities offer numerous opportunities for potential cross-contamination and the introduction of *Listeria* onto the shell of eggs. There are two types of egg-processing facilities, in-line and off-line [58]. In-line processing occurs at the same location as egg production, with the eggs being delivered directly for processing by an automated conveyor system [58]. In the off-line processing model, eggs are produced at satellite farms and delivered to the processing facility via trucks [58]. Funk [59] conducted egg cooling studies and determined that when individual eggs with initial temperatures from 33 C to 39 °C were placed in a cooler (10 °C), the eggs cooled by approximately 11 °C per hour. When placed in the cooler in wire baskets, eggs in the center lost 4 °C per hour as opposed to eggs in metal buckets, which cooled at half the rate [59]. Shell eggs can be produced in either an off-line or on-line facility; in an off-line facility, the hens are housed in one location, and the eggs are transported to the processing facility as opposed to in-line, where the hen houses are directly connected to the processing facility.

In the U.S., eggs must be washed and sanitized before being sold as shell eggs or sent to a breaker for further processing [24]. USDA requires that shell eggs be washed in water that is at least 20 °F (11.1 °C) warmer than the warmest egg on the processing line but not less than 90 °F (32.2 °C), followed by a rinse with an approved sanitizer at the same or higher temperature as the wash water [24]. Jones et al. [60] investigated three washing schemes for shell eggs using two separate washers; the washers used (1) hot water in both washers (HH), (2) hot water then cold water (HC), or (3) cold water in both washers (CC).

Manfreda et al. [61] developed a system using hot air to decontaminate shell eggs. The eggs were positioned on rolling cylinders with two hot air generators above and a cold air generator below. As a treatment, the eggs were given two 8-second bursts of 600 °C air while cold air (20 to 25 °C) was blown from below; the bursts of hot air were given 32 s apart [61]. *L. monocytogenes* was reduced by 1.2 logs CFU/eggshell immediately after treatment. Eggs were stored at 20 to 25 °C for 28 days; *L. monocytogenes* numbers were below the detection limit on both treated and untreated eggs at the end of this storage period [61].

## 7. *Listeria* and Retail Shell Eggs

After washing, eggs are subjected to "candling", which involves inspecting the interior of the egg as it is rotated over a bright light to determine if there are any cracked or dirty shells and ensure that the yolk and albumen are in good shape and that there are no blood or meat spots visible [58]. Eggs destined to be sold as shell eggs undergo packaging. Eggs can be "loosely packed", which means they are placed in a 20- or 30-egg flat to be sold primarily to restaurants [58]. For eggs sold directly to consumers, some cartons hold 12 or 18 eggs; these cartons are made attractive to appeal to the customer [62]. Eggs intended for further processing are sent to breakers where the liquid contents are separated from the shells to be sold as liquid whole eggs (LWE), liquid and dried whites, and liquid and dried yolks [58].

Once the eggs leave the processing facility, they are placed in refrigeration, where any *L. monocytogenes* contamination already present could continue to proliferate [63]. Guzmán-Gómez et al. [64] obtained shell eggs of five commercial brands from ten retail outlets and compared the results of standard culture techniques with those from nested PCR (n-PCR). Three of the eggshell samples tested positive by PCR for *L. monocytogenes,* while none were positive on culture, leading the authors to suggest that more sensitive methods, such as the n-PCR, should be used as a standard method rather than relying on the culture technique [64].

This risk is particularly evident if minimal cooking is involved. For example, retail eggs may subsequently be consumed raw by the consumer in the form of protein shakes, or they may be used in additional processing and consumed in the form of raw cookie dough [21]. However, even if the table eggs are purchased by the consumer and subsequently cooked, this may not be sufficient to kill the bacterium [65]. Brackett and Beuchat [65] added $10^2$ CFU/g and $10^5$ CFU/g *L. monocytogenes* to eggs, fried the eggs "sunnyside up", and scrambled the eggs to an internal temperature of 70 to 73 °C. Only a 0.4 log decrease was observed when frying the eggs "sunny side up" while scrambling the eggs resulted in a 3 log reduction or, in the case of the low contamination event, brought populations below the limit of detection (1 CFU/g). These data suggest that *Listeria* can survive the cooking process despite the 3 log reduction observed by scrambling.

## 8. Recent Developments for Interventions for *Listeria* on Shell Eggs

In the U.S., the USDA requires that shell eggs must be sanitized by a rinse equivalent to 100 to 200 ppm chlorine or the equivalent [66]. However, participants in the National Organic Program certification (NOP) are required to remove all chlorine compounds on the surface of organic products with a potable water rinse, increasing costs and waste for the farmer [24]. However, in the EU, eggs are not washed, and the use of any disinfecting rinse causes the eggs to be downgraded [67]. The use of ultraviolet (UV) light to decontaminate the surface of eggs has become an area of interest; UV light is lethal to most microorganisms because of the damage to nucleic acids (DNA and RNA) [68]. Unfortunately, some microorganisms can repair this damage and become viable again; therefore, UV treatment must be administered to completely disrupt the nucleic acid [68][69]. The use of UV-C light for decontamination of food products is allowed in both the U.S. and the E.U., although Germany limits the use of UV-C to water, produce, and hard cheeses [70].

Electrostatic sprayers have also gained attention; the electrostatic sprayer works by using a positively charged surface to attract the negatively charged fluid droplets, which assures an even coating of the surface and minimizes exposure time [71]. Russell [72] used an electrostatic sprayer to spray electrolyzed oxidative (EO) water onto eggs artificially contaminated with *L. monocytogenes*. The treatment reduced *L. monocytogenes* below detection limits on 8 (53.3%), 13 (86.7%), 12 (80%), and 14 (93.3%) eggs of 15 tested in four separate replications [72].

## 9. *Listeria* and Liquid Egg Products

Approximately 30% of eggs produced in the U.S. are sent to "breakers" to be processed into liquid or dried products, including liquid whole egg (LWE), liquid or dried yolks, and liquid or dried whites [73]. Many breakers are in-line, receiving eggs via a conveyor belt from the receiving area, which is connected directly to the hen houses, although some may be off-line, receiving eggs via truck from satellite farms [73]. Eggs move to a breaking machine where eggs are individually grasped and broken to let the liquid contents drain into a trough, which leads to a filter and then into a holding tank where the LWE is cooled as rapidly as possible to 4 °C; pasteurization and packaging are the final steps [73].

Leasor and Foegeding [74] collected raw LWE samples from egg processors in 11 states in the U.S. over eight months. When cultured for the presence of *Listeria* spp., 15 of the 42 samples (36%) were positive for *Listeria*; *L. innocua* was isolated from all the positive samples, while *L. monocytogenes* was isolated from two samples [74]. Rivoal et al. [75] used pulsed-field gel electrophoresis (PGFE) to determine if *L. monocytogenes* was present in raw or pasteurized LWE after storage at 2 °C for 2 days and at the end of shelf life. They detected *L. monocytogenes* in 25 of 144 raw samples, 4 in the pasteurized sample, and 2 of 144 that were at the end of shelf life.

Liquid eggs can be combined from as many as 15 to 20 eggs into a one-liter product [76]. Pasteurization of liquid eggs can be used to improve product safety [77]. Li et al. [78] investigated two liquid egg products (A and B) with a water activity of 0.76 and 0.82 and a viscosity of 183 and 119 centipoise/s, respectively. After inoculation with $10^9$ log CFU/mL, egg samples were subjected to 64, 66, 68, and 70 °C. At 70 °C, a D value of 0.133 min for product A and 0.74 min for product B was determined. At 64 °C, a D value of 0.440 min for product A and 0.364 min for product B was determined. These values were higher than for *Salmonella* at all temperatures, with 70 °C D-values being 0.035 min (product A) and 0.048 min (product B).

## 10. *L. monocytogenes* in Ready-to-Eat (RTE) Egg Products

Most ready-to-eat (RTE) egg products, such as egg patties, omelets, and scrambled eggs, are fully cooked and reach temperatures of 85 °C (185 °F) before packaging and freezing [79]. Since *L. monocytogenes* is adequately inactivated by cooking to an internal temperature of 70 °C (158 °F) for 2 min, this treatment is adequate [80]. However, there is still the possibility of postprocess contamination with *L. monocytogenes,* plus the consumer preference for unfrozen products, which increases the food safety risk.

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