Prevalence of coliforms, *Salmonella*, *Listeria*, and *Campylobacter* associated with eggs and the environment of conventional cage and free-range egg production

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ABSTRACT There is a desire by US consumers for eggs produced by hens in alternative production systems. As the retail shell-egg market offers these products to accommodate consumer demands, additional information is needed to ensure processing methodologies result in safe eggs from all egg sources. A study was conducted to determine if there were differences in the prevalence of coliforms, *Salmonella*, *Listeria*, and *Campylobacter* on and within eggs and in the environment of a sister flock of conventional cage and free-range laying hens. Microbial sampling occurred approximately every 6 wk between 20 and 79 wk of age. A random sampling of typical coliform colonies produced 371 viable isolates for biochemical identification. Twenty-nine genera or species of bacteria were identified. There was a significantly greater ($P < 0.0001$) prevalence of *Campylobacter* in the free-range nest box swabs compared with that in the free-range grass and conventional cage swab samples (number of positives: 8 nest box, 1 grass, 0 cage). Seven isolates of *Listeria innocua* were detected with no significant difference in prevalence between the treatments. Isolates were associated with eggshells (2 free-range floor, 1 cage) and the free-range environment (2 nest box, 2 grass). There were 21 *Salmonella* isolates detected between all sample locations, with no significant difference in the prevalence of *Salmonella* detection between the treatments. Additional studies are needed to fully understand the effect of alternative production methods on the prevalence of pathogens and coliforms associated with nest-run eggs and the production environment.

Key words: egg, housing, *Salmonella*, *Campylobacter*, *Listeria*

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INTRODUCTION

Determining the *Salmonella* status, with particular emphasis on *Salmonella enterica* serovar Enteritidis, of egg production flocks and eggs in commerce has been a topic of scientific concern for many years. In 2009, the US Food and Drug Administration published a Final Rule defining parameters for reducing the incidence of *Salmonella* Enteritidis in eggs during production, storage, and transportation (FDA, 2009). Researchers have monitored the effect of various hen management and nutritional practices on *Salmonella* colonization and proliferation in laying hens (Farnell et al., 2001; Jones et al., 2002; Golden et al., 2008; Gutierrez et al., 2008; Callaway et al., 2009). Additionally, other pathogenic organisms, such as *Campylobacter*, *Escherichia coli*, and *Listeria*, have been detected in unwashed eggs entering shell egg and egg product processing facilities (Neill et al., 1985; Allen and Griffiths, 2001; Jones and Musgrove, 2007; Musgrove et al., 2008).

Alternative housing systems for hens have become a consideration for consumers and legislatures around the world. Legislations defining egg production practices have been enacted in Europe and the United States with effective dates of 2012 and 2015, respectively (European Council, 1999; California Health and Safety Code, 2009). Other legislative bodies have begun discussions of animal husbandry practices that could lead to legislations of various forms. Scientific publications assessing the effects of various hen housing conditions on environmental and egg microbiology are contradictory. A white paper comparing peer-reviewed assessments of *Salmonella* contamination, with particular emphasis on *Salmonella* Enteritidis, in regards to hen housing conditions has been published (Holt et al., 2011). The authors concluded that no clear recommendations can be made due to the variable nature of study conditions (breed of hens, hen age, environment, housing system

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age, hen diet, climate, management practices, and so on).

The current study was undertaken to compare the effects of conventional cage and free-range production on environmental and egg microbiology. A sister flock of pullets were split between the 2 housing environments on a single commercial-style research farm while being fed the same diet. The controlled nature of the study allows for direct comparison between the 2 production environments. The general microbiological differences of the study have been reported (Jones et al., 2011). The current study presents the differences in pathogen detection between the 2 housing environments.

MATERIALS AND METHODS

Hen Management

A flock of Hy-Line Brown hens was hatched at the Piedmont Research Station, North Carolina Department of Agriculture and Consumer Services, in Salisbury. The full description of rearing and production management was reported by Anderson (2008). Briefly, all chicks were housed in the same brood/grow pullet house equipped with conventional cages or floor pens. The chicks to be used for conventional cage production were reared in a quad-deck system with 13 birds/cage (310 cm^2/bird). The chicks for free-range production were reared in floor pens on litter (929 cm^2/bird) with access to roosts. At 12 wk of age, the floor-reared hens were moved to the range environment to complete the rearing phase.

At 17 wk of age, the conventional cage-reared hens were moved to a quad-deck laying house with 4 cage replicates of 6 hens/cage (413 cm^2/hen) for a replicate population of 24 hens. For free-range production, 75 hens were housed in each range hut/paddock, equating to 929 cm^2/hen in the range hut, 13 cm of roosting space/hen, and 1 nest box/8 hens. The range paddock afforded 8.04 m^2 of forage area per hen. All dietary and lighting regimens were equivalent and are detailed by Anderson (2011).

Environmental and Egg Sample Collection

Environmental and egg samples were collected from conventional cage, free-range nest box, and free-range grass approximately every 6 wk from 20 to 79 wk of hen age (11 sampling periods). Swabs were collected (in triplicate) from the conventional cage wire egg collection area (**CS**) and free-range nest boxes (**FRNS**) using a 10- × 10-cm sterile gauze pad moistened with 20 mL of sterile PBS. After swabbing, each gauze pad was placed in a sterile sample bag and transported to the laboratory on ice. Free-range grass (**FRG**) samples were aseptically collected using sterile shears to cut a handful of grass 2.5 cm from the ground. The grass samples were placed into sterile sample bags and transported to the laboratory on ice. These sample sites were selected because they were egg contact surfaces. Furthermore, grass from the paddock area provides an indication of hen environmental microbial exposure. All samples were stored at 4°C overnight before analysis.

The following morning, 30 mL of sterile PBS was added to each swab sample and stomacher-blended (Stomacher 400 Circulator, Seward Ltd., London, UK) for 1 min at normal speed (230 rpm). Grass samples were aseptically cut into small pieces with sterile shears. Grass samples were then weighed and sterile PBS was added to the samples at a 1:10 ratio. Samples were then stomacher-blended for 1 min at normal speed.

A 30-egg flat for each treatment [conventional cage (**CC**), free-range nest box (**FRNB**), and free-range floor (**FRF**)] was aseptically collected at the research farm. The CC and FRNB were laid in roll-out style cages and nest boxes, respectively, that allow eggs to roll out into a collection tray. Eggs from each treatment were placed into a clean laboratory bag and transported back to the laboratory on ice and stored at 4°C overnight. The following morning, cracked eggs were discarded. For each treatment, 8 pools of 3 eggs each were formed for both shell emulsions and egg contents. Shell emulsion pools were compiled in sterile specimen cups, according to the methods of Musgrove et al. (2005), using 50 mL of 42°C sterile PBS. Egg content pools were formed in sterile laboratory sample bags and stomacher-blended for 1 min at normal speed according to the methods of Jones et al. (2004).

Microbial Analyses

Detection of *Campylobacter*, *Listeria*, and *Salmonella* was conducted according to the methods of Jones et al. (2006). Briefly, for *Campylobacter* determination, a 10-mL aliquot of sample was enriched in Bolton's broth (*Campylobacter* enrichment broth, Acumedia Manufacturers, Lansing, MI; Bolton broth selective supplement, Oxoid Limited, Basingstoke, UK; defibrinated horse blood, Lampire Biological Laboratories, Pipersville, PA) under modified atmosphere. One-tenth of a milliliter was subsequently exposed to Campy Cefex plates (Stern et al., 1995) incubated under modified atmosphere. Suspect colonies were confirmed via wet-mount microscopic examination and latex agglutination (Microbiology International, Frederick, MD).

A 10-mL aliquot of sample was introduced into UVM modified *Listeria* enrichment broth (Acumedia Manufacturers) to initiate *Listeria* pre-enrichment. This was followed with Fraser broth enrichment (broth, Acumedia Manufacturers; supplement, Becton Dickinson, Sparks, MD). Selective plating on modified Oxford (Becton Dickinson) was then conducted. Presumptive positive colonies were introduced onto motility agar (Acumedia Manufacturers). Presumptive positive colonies were identified biochemically (Microgen *Listeria* ID kit, Microbiology International).

The remaining samples, not used for general microbial enumeration (Jones et al., 2011), were pre-enriched

Table 1. Seasonal influence of *Campylobacter* prevalence (no. positive/total no.) in shell emulsion pools of conventional and free-range egg production

Season[1]	Conventional cage	Free-range nest box	Free-range floor
Spring	0/16	0/16	0/15
Summer	0/16	2/16	0/15
Fall	1/40	0/39	0/33
Winter	0/16	0/16	0/11
P-value	NS	0.05	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

with buffered peptone water (Accumedia Manufacturers) to initiate *Salmonella* detection. Aliquots (0.1 mL) were then enriched in both Rappaport-Vassiliadis (Becton Dickinson) and tetrathionate Hajna (Becton Dickinson) broths. Each enriched sample was then plated on both brilliant green sulfa (Acumedia Manufacturers) and XLT4 (agar, Acumedia Manufacturers; supplement, Becton Dickinson). Presumptive positive colonies were stabbed on both lysine iron agar (Becton Dickinson) and triple sugar iron agar (Becton Dickinson). Colonies presenting *Salmonella* properties were subjected to agglutination (Microbiology International).

Confirmation of Salmonella Serotype

Salmonella serotype was determined by PCR amplification of the *dkgB*-linked intergenic spacer ribosome (**ISR**) region to obtain the sequence from the first base pair after the 23S ribosomal gene to the last base pair before tRNA aspU (Morales et al., 2006). It includes the entire 5S ribosomal gene. The ribosomal area of interest is linked to *dkgB*, which was previously named *yafB*. To obtain DNA, single colonies were grown in 10 mL of brain heart infusion broth (Becton Dickinson) at 37°C for 16 h. Bacterial cells were pelleted in an RC5B Plus centrifuge at 5,000 × *g* for 15 min with a Superlite SLA 600TC rotor (Sorvall, Thermo Fisher Scientific, Waltham, MA). For confirmatory sequencing, total DNA was extracted using a Genomic-tip 100/G kit following the protocol designated for bacteria (Qiagen, Valencia, CA). Precipitated DNA was dissolved in 200 μL of Tris-EDTA buffer [10 m*M* Tris-HCl and 1 m*M* EDTA (pH 8)] and stored at −20°C. Spectrometer readings of DNA samples were obtained using a NanoDrop 1000 (Wilmington, DE) to ensure optical density 260/280 ratios were greater than 1.7 and that DNA concentration was above 20 ng/μL. To determine serotype, the sequence trimmed to the aforementioned ISR region was aligned to reference sequences deposited at NCBI (http://www.ncbi.nlm.nih.gov/) by DNASTAR Lasergene SeqMan (version 8.0.2, Madison, WI) using default project assembling parameters except as follows: minimum match percentage 100 and minimum sequence length 100. Only perfect matches can be used to call serotype. The ISR reference sequences that define serotype have GenBank accession numbers JN105119-JN105125 and JN092293-JN092328 (release date 8/1/2011). All primers required for PCR amplification and sequencing have been deposited at NCBI in association with ISR sequences.

Biochemical Identification of Coliform Isolates

Enumeration of coliforms from the samples has been previously reported (Jones et al., 2011). Up to 5 typical colonies per positive sample were randomly selected from violet red bile agar plates and struck for isolation on standard methods agar (Acumedia Manufacturers). Two additional passes on standard methods agar were conducted to ensure purity of isolates. Single colonies were then transferred to Biolog BUG agar plus 5% sheep's blood (Biolog, Hayward, CA) and incubated overnight at 37°C. After incubation, colonies were tested to determine oxidase activity and gram status, according to the manufacturer's recommended procedures. Then, fresh cells were harvested from the BUG plus blood plate and inoculated into the prescribed testing diluent for the identification kit. Samples were then placed on the appropriate microplate system (Bi-

Table 2. Seasonal influence of *Campylobacter* prevalence (no. positive/total no.) in the environment of conventional and free range egg production

Season[1]	Conventional cage swab	Free-range nest box swab	Free-range grass
Spring	0/6	0/6	0/6
Summer	0/6	3/6	0/6
Fall	0/14	4/14	1/15
Winter	0/6	1/6	0/6
P-value	NS	NS	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

Table 3. Seasonal influence of *Listeria* prevalence (no. positive/total no.) in shell emulsion pools of conventional and free-range egg production

Season[1]	Conventional cage	Free-range nest box	Free-range floor
Spring	0/16	0/16	0/15
Summer	0/16	0/16	0/15
Fall	1/40	0/39	2/33
Winter	0/16	0/16	0/11
P-value	NS	NS	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

olog) and loaded into the Omniolog (Biolog) for the identification procedure.

Statistical Analysis

Before analysis, sampling periods were classified according to the season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer. According to this classification, the number of sampling periods per season was 5 for the fall, 2 for the winter, 2 for the spring, and 2 for the summer. Treatment and season were the main effects. The Chi-squared operation and goodness of fit test were used to assess frequency data for pathogen detection. Probabilities of $P < 0.05$ were considered significant. All statistical analyses were conducted using SAS software (SAS Institute, 2002).

RESULTS

Campylobacter was detected in each housing situation at least once (Figure 1). *Campylobacter*-positive shell emulsion pools were detected in CC and FRNB. There was a significant difference ($P < 0.05$) in the frequency of detection for FRNB between seasons (Table 1). Both positive FRNB shell emulsion pools were found during the spring (12.5% of spring samples positive). The single positive shell emulsion sample detected in CC was found during the fall and represented 2.5% of the total fall samples collected. There was no seasonal difference for *Campylobacter* detection frequency in shell emulsions for CC. No *Campylobacter* was detected in shell emulsions of FRF eggs.

All egg contents tested during the study were negative for *Campylobacter* (n = 249; Figure 1). There was a significantly greater occurrence (25% of samples; $P < 0.05$) of *Campylobacter* detection in FRNS compared with CS (none detected) and FRG (3%; Figure 1). There was no significant difference ($P > 0.05$) in the frequency of detection for FRNS based on season (Table 2). The highest rate of detection occurred in the summer (50%), followed by fall (29%) and winter (17%). A single FRG sample was positive for *Campylobacter* in the fall.

A low frequency of *Listeria* detection occurred during the study (Figure 2). Although *Listeria* was found at

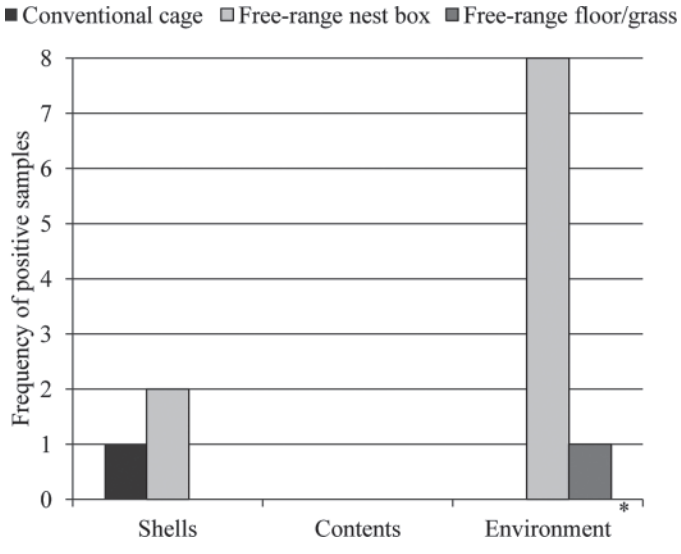

Figure 1. Frequency of *Campylobacter* detection in conventional and free-range egg production. Conventional cage: shells, n = 88; contents, n = 88; environmental swabs, n = 32. Free-range nest box: shells, n = 87; contents, n = 87; environmental swabs, n = 32. Free-range floor: shells, n = 74; contents, n = 74; environmental grass, n = 33. *Significant difference among treatments, $P < 0.05$.

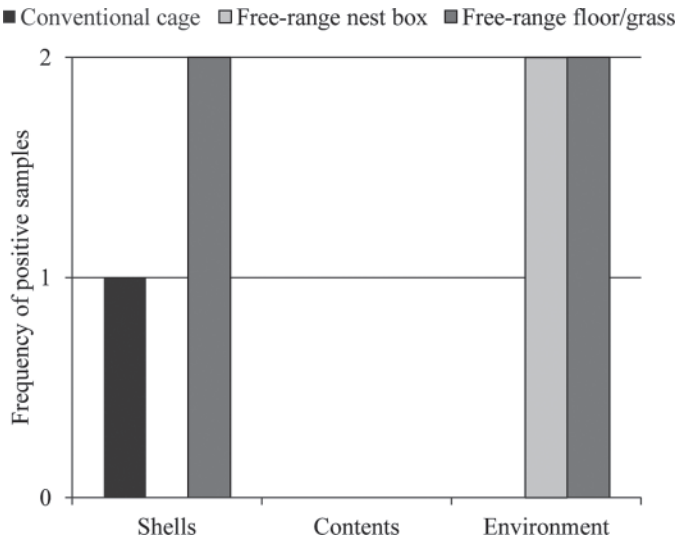

Figure 2. Frequency of *Listeria* detection in conventional and free-range egg production. Conventional cage: shells, n = 88; contents, n = 88; environmental swabs, n = 32. Free-range nest box: shells, n = 87; contents, n = 87; environmental swabs, n = 32. Free-range floor: shells, n = 74; contents, n = 74; environmental grass, n = 33.

Table 4. Seasonal influence of *Salmonella* prevalence (no. positive/total no.) in shell emulsion pools of conventional and free-range egg production

Season[1]	Conventional cage	Free-range nest box	Free-range floor
Spring	0/16	0/16	0/15
Summer	0/16	0/16	0/15
Fall	0/40	0/39	3/33
Winter	4/16	0/16	0/11
P-value	0.05	NS	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

least once in all treatments, none was detected in any of the egg contents pools (n = 249). In the shell emulsion pools, there were 3 samples positive for *Listeria* (1 CC and 2 FRF). All 3 of the pools contained *Listeria innocua* and were collected during the fall (Table 3). There were no significant seasonal differences ($P > 0.05$) in *Listeria* detection for either CC or FRF shell emulsions. Two FRG samples, both from a single summer collection, contained *Listeria monocytogenes*. Two FRNS contained *L. innocua* (spring and winter).

Salmonella was detected in all treatments (Figure 3). There were no significant differences ($P > 0.05$) in detection frequency among the treatments for shell emulsion or egg contents pools as well as environmental samples. *Salmonella* was not detected in shell emulsion pools for FRNB eggs (n = 87). All *Salmonella* isolates from CC shell emulsions were found during the winter (25%; $P < 0.05$; Table 4). All isolates from FRF shell emulsions were detected in the fall (9%). *Salmonella* was the only pathogen monitored during the study that was detected in egg contents. Furthermore, it was found in the contents of all treatments (Figure 3). The 2 positive samples from CC eggs were detected in the summer (13%; $P < 0.05$; Table 5). The 3 occurrences from FRNB eggs were also detected in the summer (19%; $P < 0.05$). The 2 contents pools from FRF were collected in the fall (6%; $P > 0.05$).

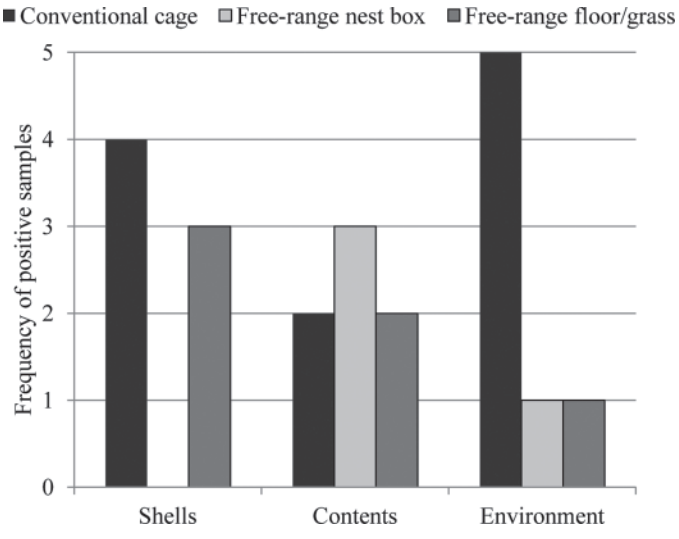

Figure 3. Frequency of *Salmonella* detection in conventional and free-range egg production. Conventional cage: shells, n = 88; contents, n = 88; environmental swabs, n = 32. Free-range nest box: shells, n = 87; contents, n = 87; environmental swabs, n = 32. Free-range floor: shells, n = 74; contents, n = 74; environmental grass, n = 33.

All treatments had a least one *Salmonella*-positive environmental sample (Figure 3). The greatest number of positive environmental samples occurred in CS where at least one was found in each season (Table 6). A single positive sample was found in both FRNS and FRG, with both occurring in the fall. Of the 21 *Salmonella* isolates collected throughout the study, across all treatments and sample types, 20 were molecularly serotyped. (There was a single PCR failure resulting in no identification.) Eighteen of the isolates were *Salmonella* Typhimurium. One isolate was *Salmonella* Javiana (FRF shell emulsion). A single isolate was *Salmonella* Enteritidis (FRG).

A total of 359 coliform isolates were biochemically identified as 29 different organisms (Table 7) from all sample types and treatments. The greatest number of isolates (224 isolates; 62%) was collected from shell emulsion pools, primarily from free-range production. The fewest isolates (28 isolates; 8%) were from egg contents pools. The primary isolate (196 isolates; 55%) was *Escherichia coli*, with the greatest number of these coming from FRNB shell emulsions (87 isolates; 44%). *Escherichia coli* was cultured from all sample types and treatments, except for CC contents. *Escherichia* spp. represented 57% of the total number of isolates. The second most frequent isolate was *Pantoea agglomerans* (52 isolates; 14%), with no isolates from egg contents samples. *Pantoea* spp. represented 18%, the second highest number of isolates. *Citrobacter braakii*, *Enterobacter amnigenus*, *Enterococcus faecium*, *Escherichia fergusonii*, *Lactococcus lactis*, and *Leclercia adecarboxylata* were identified multiple times only in free-range production samples. *Enterococcus faecium* was isolated from FRF and FRNB egg contents samples. *Escherichia fergusonii* was identified from FRNB shell emulsions and FRNS. *Lactococcus lactis* isolates were all from FRF egg contents samples.

DISCUSSION

Efforts have been made to better understand the presence of *Campylobacter* in laying hens, such as fecal swabs (Sulonen et al., 2007; Dipineto et al., 2011) and organs (Cox et al., 2009), to determine prevalence. Sulonen et al. (2007) collected fecal samples in the spring and fall from Finnish organic egg farms. In the fall, 84%

Table 5. Seasonal influence of *Salmonella* prevalence (no. positive/total no.) in egg contents pools of conventional and free-range egg production

Season[1]	Conventional cage	Free-range nest box	Free-range floor
Spring	0/16	0/16	0/15
Summer	2/16	3/16	0/15
Fall	0/40	0/39	2/33
Winter	0/16	0/16	0/11
P-value	0.05	0.05	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

of farms were positive for *Campylobacter*; whereas 76% were positive in the spring. Differing genotypes of *Campylobacter* were detected between the spring and fall. The prevalence of *Campylobacter* within the samples from a single flock ranged from 5 to 100%. Dipineto et al. (2011) determined the prevalence of *Campylobacter* on 4 intensive-management farms in Italy. *Campylobacter* was isolated from 65.3% of samples. A greater recovery was found in the summer versus winter collections. Unlike the current study, both of these studies sampled exclusively during 2 seasons, preventing a complete understanding of potential seasonal effects of *Campylobacter* detection. Although the current study did not include fecal samples, there was an issue with hens defecating in the nest boxes that could account for the significantly higher level of *Campylobacter* detection between the environmental samples. As is the case for the current study, Sulonen et al. (2007) reported a very low detection rate of *Campylobacter* on shells (1 out of 360). The findings of Dipineto et al. (2011) concur with the current study in reporting no *Campylobacter*-positive egg contents.

The prevalence of *Listeria* in the current study was very low. Schwaiger et al. (2010) compared cloacal swabs from 20 conventional and organic egg farms in Germany, resulting in very low *Listeria* prevalence (1.8% and 1.3%, respectively) with no significant difference between production methods. Conversely, Aury et al. (2011) cultured fecal samples from 84 conventional cage farms in France, resulting in a *Listeria monocytogenes* prevalence of 31%. Two free-range grass samples were positive for *L. monocytogenes* in the current study, with 2 nest-box swabs containing *L. innocua*. In the current study, 3 shell emulsion pools (1 CC, 2 FRF) contained *L. innocua*; no *Listeria* was detected in any egg contents pools. Schwaiger et al. (2010) detected *L. seeligeri* in a single conventional cage egg contents pool and no *Listeria* in any shell pools. The diverse results from these studies illustrate the need for additional research before a complete understanding of hen management and *Listeria* prevalence can be drawn.

In the current study, there were no significant differences in *Salmonella* prevalence among the hen housing conditions for any of the sample types, even though *Salmonella* was the only monitored pathogen detected in all treatments. Even though free-range hens had access to outdoors and more contact with their eggs, *Salmonella* prevalence was not greater, which is similar to the results of De Vylder et al. (2009), who found no indication that alternative housing increased *Salmonella* contamination. Huneau-Salaün et al. (2009) and Van Hoorebeke et al. (2010) monitored commercial laying flocks in Europe, generally via fecal samples, reporting a greater likelihood of *Salmonella* prevalence in battery cage production. In the current study, the greatest number of *Salmonella*-positive shell emulsion and environmental swabs were found in CC production. Conventional cage production had the most dust present on the eggs and on the equipment surfaces. Gast et al. (1998) have shown the downstream movement of *Salmonella* Enteritidis from inoculated chicks to feathers of control chicks, at a 77% prevalence rate. Additionally, Huneau-Salaün et al. (2009) stated the risk of detecting *Salmonella* Enteritidis is greater in dust versus fecal samples. Therefore, the presence of dust in the current study could play a role in the current outcomes. As with Huneau-Salaün et al. (2009), *Salmonella* Typhimurium was the most frequently isolated species in the current study. Van Hoorebeke et al. (2011) declared that the underlying mechanisms causing *Salmonella* prevalence to be lower in alternative housing remains unknown. The authors concur that more research is

Table 6. Seasonal influence of *Salmonella* prevalence (no. positive/total no.) in the environment of conventional and free-range egg production

Season[1]	Conventional cage swab	Free-range nest box swab	Free-range grass
Spring	1/6	0/6	0/6
Summer	1/6	0/6	0/6
Fall	2/14	1/14	1/15
Winter	1/6	0/6	0/6
P-value	NS	NS	NS

[1]Season of the year based on astronomical classification with the seasons beginning: September 21 = fall; December 21 = winter; March 21 = spring; and June 21 = summer.

Table 7. Identification of coliform isolates from conventional and free-range egg production[1]

Coliform	Total no.	Shell emulsion			Contents			Environment		
		FRF	FRNB	CC	FRF	FRNB	CC	FRG	FRNS	CS
Aeromonas spp.	1							1		
Buttiauxella izardii	1	1								
Cedecea davisae	5	3						1		1
Citrobacter braakii	8	3	1					1	3	
Citrobacter sedlakii	3	3								
Enterobacter aerogenes	2									2
Enterobacter amnigenus	13	6	4			1		2		
Enterobacter asburiae	1			1						
Enterobacter cancerogenus	1							1		
Enterobacter nimipressuralis	3	1		2						
Enterococcus faecium	4				2	2				
Escherichia coli	196	34	87	17	1	7		11	35	4
Escherichia fergusonii	3		2						1	
Escherichia vulneris	6						4	1	1	
Klebsiella oxytoca	1									1
Klebsiella pneumoniae	2			1						1
Lactococcus lactis	8				8					
Leclercia adecarboxylata	5	1	2		1				1	
Pectobacterium carotovorum	1							1		
Pectobacterium cypripedii	1								1	
Pantoea spp.	1			1						
Pantoea agglomerans	52	19	10	3				11	1	8
Pantoea dispersa	11	3	1	2	1			2		2
Pantoea stewartii	1	1								
Rahnella aquatilis	2					1		1		
Raoultella terrigena	21	7		6				3	1	4
Serratia marcescens	4							4		
Serratia proteamaculans	1	1								
Shigella flexneri	1	1								
Total	359	84	107	33	13	11	4	40	44	23

[1]FRF = free-range floor; FRNB = free-range nest box; CC = conventional cage; FRG = free-range grass; FRNS = free-range nest box swab; and CS = conventional cage wire swab.

needed to determine the effect of various housing conditions on *Salmonella* prevalence associated with eggs and in the production environment.

Escherichia coli were the predominant coliforms isolated in the current study. Schwaiger et al. (2008) found the greatest amounts of *E. coli* in fecal samples but relatively low numbers associated with eggshells. Conversely, the greatest percentage of *E. coli* isolates in the current study was from eggshells. It is almost impossible to draw clear parallels and differences between the current study and the results presented by Schwaiger and colleagues (2008), in part because of differences in sampling and climate. Most of the identified organisms in both studies are ubiquitous to nature and are associated with feces. As such, climate will have a direct effect on their presence and proliferation.

Current US shell egg washing procedures are designed to clean eggs from CC production. Although additional work is needed to completely assess any differences in baseline egg microbiology between conventional and alternative production methods, trends occur in the current study. The greatest percentage of identified isolates came from shell emulsion pools. Only 15% of these isolates were from CC production. Not only did a greater number of isolates come from free-range eggshells, a larger diversity in organisms was also seen. Ten organisms were found in free-range shell emulsions and not conventional shells. Hens in free-range production are exposed to external microbial influences, such as soil, feces, wild animals, and rodents, not found in CC production. While the current study, and those referenced, begin to determine the effects of conventional and alternative production methods on environmental and egg microbiology, much more work is needed to gain a full understanding of the microbial implications.

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