Influence of commercial laying hen housing systems on the incidence and identification of *Salmonella* and *Campylobacter*[1]

D. R. Jones,[*,2] J. Guard,[*] R. K. Gast,[*] R. J. Buhr,[*] P. J. Fedorka-Cray,[*,3] Z. Abdo,[*] J. R. Plumblee,[*] D. V. Bourassa,[*] N. A. Cox,[*] L. L. Rigsby,[*] C. I. Robison,[†] P. Regmi,[†] and D. M. Karcher[†]

[*]*USDA, Agricultural Research Service, US National Poultry Research Center, Athens, GA 30605; and*
[†]*Department of Animal Science, Michigan State University, East Lansing, MI 48824*

ABSTRACT The housing of laying hens is important for social, industrial, and regulatory aspects. Many studies have compared hen housing systems on the research farm, but few have fully examined commercial housing systems and management strategies. The current study compared hens housed in commercial cage-free aviary, conventional cage, and enriched colony cage systems. Environmental and eggshell pool samples were collected from selected cages/segments of the housing systems throughout the production cycle and monitored for *Salmonella* and *Campylobacter* prevalence. At 77 wk of age, 120 hens per housing system were examined for *Salmonella* and *Campylobacter* colonization in the: adrenal glands, spleen, ceca, follicles, and upper reproductive tract. All isolates detected from environmental swabs, eggshell pools, and tissues were identified for serotype. Two predominant *Salmonella* were detected in all samples: *S.* Braenderup and *S.* Kentucky. *Campylobacter coli* and *C. jejuni* were the only *Campylobacter* detected in the flocks. Across all housing systems, approximately 7% of hens were colonized with *Salmonella*, whereas > 90% were colonized with *Campylobacter*. *Salmonella* Braenderup was the isolate most frequently detected in environmental swabs ($P < 0.0001$) and housing system impacted *Salmonella* spp. shedding ($P < 0.0001$). *Campylobacter jejuni* was the isolate most frequently found in environmental swabs ($P < 0.01$), while housing system impacted the prevalence of *C. coli* and *jejuni* in ceca ($P < 0.0001$). The results of this study provide a greater understanding of the impact of hen housing systems on hen health and product safety. Additionally, producers and academia can utilize the findings to make informed decisions on hen housing and management strategies to enhance hen health and food safety.

Key words: hen housing systems, egg, *Salmonella*, *Campylobacter*, colonization

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INTRODUCTION

Laying hen housing for commercial egg production continues to be a topic of social and political discussions in the US. Commercial housing systems have become available allowing egg producers options to meeting consumer and regulatory demands. Researchers have been working to determine the best options for managing hens in non-conventional cage systems to optimize hen health and well-being, while also efficiently producing safe, high quality eggs (De Reu et al., 2005, 2006, 2009; Mallet et al., 2006; Schwaiger et al., 2008).

The impact of hen housing on egg safety and hen health is complex due to the variables involved (Holt et al., 2011). As part of the critical review of literature available, Holt and colleagues determined that while Europe had conducted many explorations of hen housing impact on egg safety, the results were often conflicting. Many hen genetic, nutrition, management, and egg handling practices utilized in Europe are not applicable in the US and vice versa, necessitating further US hen housing research. Since that time, additional findings from the US have been reported, but conflicting results are still common, in part due to the dynamic nature of animal husbandry (Hannah et al., 2011; Jones et al., 2011, 2012, 2015; Gast et al., 2013, 2014; Jones and Anderson, 2013).

An extensive collaborative comparison of commercial conventional cage, enriched colony cage, and cage-free aviary hen housing systems has been conducted by a team of researchers in the US. Five sustainability areas were investigated: hen health and well-being, environmental impact, food safety and quality, food affordability, and worker health and safety (Jones et al.,

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[2]Corresponding author: Deana.Jones@ars.usda.gov
[3]Present address: Department of Population Health and Pathobiology, North Carolina State University, Raleigh, NC 27695

2014, 2015; Arteaga et al., 2015; Karcher et al., 2015; Matthews and Sumner, 2015; Mitchell et al., 2015; Regmi et al, 2015; Shepherd et al., 2015; Zhao et al., 2015a, 2015b; Blatchford et al., 2016; Campbell et al., 2016a, 2016b). The multi-year, multi-flock study has produced a large repository of information intended for egg industries, regulatory groups, and consumers to make informed decisions about commercially available hen housing systems. The current study further presents the microbiological comparisons of the housing systems. As reported by Jones et al. (2015), *Salmonella* and *Campylobacter* spp. were detected in various environmental and eggshell samples collected throughout the housing systems. The current presentation examines the identification of *Salmonella* and *Campylobacter* spp. detected. Furthermore, hens were sampled to determine the incidence and species of *Salmonella* and *Campylobacter* contamination in the tissues of hens from each of the housing systems.

MATERIALS AND METHODS

Environmental and Eggshell Pool Sample Procedures

The commercial hen housing systems management and design are described by Jones et al. (2014) and Zhao et al. (2015a), respectively. The environmental and eggshell pool sampling procedures, as well as cultural methods utilized for *Salmonella* and *Campylobacter* spp. detection are described by Jones et al. (2015). Briefly, environmental swabs were collected utilizing pre-moistened sterile sample sponges. Eggshell pools contained 6 shells (minimum of 3 shells when fewer than 6 eggs were laid in a sample location) collected from the identified cage or segment replicate. Adhering albumen was rinsed from shells with sterile phosphate buffered saline reduce impact of natural antimicrobial aspects. The number of environmental and eggshell pools collected for pathogen detection are presented in Jones et al. (2015). The low number of enriched colony cage system shell pools (n = 16 out of a possible 80) was due to the consistent use of nest boxes by the hens. The cage-free aviary production system design (Zhoa et al., 2015a) allowed for a maximum of n = 16 forage area drag swabs collected over the four sample times, although each drag swab sample was a pool of 2 swabs collected simultaneously.

Flock Termination Sample Procedures

At 77 wk of age, flock termination sampling was conducted in all three housing systems. Six hens from each of the 20 previously identified (Jones et al., 2015) microbiological sampling replicates (conventional and enriched colony cages) or segments (cage-free aviary) were randomly selected for tissue sampling (n = 120 hens per housing system). Flock termination sampling was conducted over two days with n = 60 hens sampled per housing system each day. Hens were humanely euthanized via cervical dislocation under approval of Michigan State University Institutional Animal Care and Use Committee.

Five tissue samples were aseptically collected from each hen: ceca; spleen; ovary; upper reproductive tract (infundibulum to isthmus); and adrenal gland. Samples collected each day were shipped overnight in insulated shipping boxes to the USDA Agricultural Research Service laboratories in Athens, Georgia. Immediately upon receipt, previously described *Salmonella* and *Campylobacter* enrichment procedures were initiated (Jones et al., 2015). The only exception from the previous cultural procedures was the utilization of only Rapport-Vassiliadis broth (Accumedia, East Lansing, MI) for selective *Salmonella* enrichment.

Pathogen Identification Procedures

Confirmed (via latex agglutination) *Salmonella* detected throughout the study and during flock termination tissue assessment were stored on cryobeads (Hardy Diagnostics, Santa Maria, CA) at –80°C until identification procedures were conducted. Isolates were revived on standard methods agar (Acumedia) overnight at 37°C. After overnight incubation in brain heart infusion broth (Acumedia) at 37°C, *Salmonella* serotype was determined by PCR amplification of the *dkgB*-linked intergenic spacer ribosome (ISR) region to obtain sequence from the first base pair (**bp**) after the 23S ribosomal gene to the last base pair before tRNA aspU (Guard et al., 2012). Confirmed *Campylobacter* from throughout the study and during flock termination were identified to species utilizing the *Campylobacter* BAX® PCR (DuPont Nutrition and Health, Wilmington, DE) according to manufacturer's directions. Due to a laboratory equipment failure, 104 *Campylobacter* isolates collected during production period 13 environmental and eggshell pool sampling were lost before identification was complete. The results presented in this study exclude those isolates from the calculated and analyzed percentages, and the isolate loss accounts for any discrepancies between the total number of isolates reported by Jones et al. (2015).

Statistical Analysis

The frequency of pathogen detection was analyzed by the Chi-square operation and goodness of fit test to determine significant differences (SAS Institute, 2002) with housing system and sample type as the main effects. Additionally, statistical differences in pathogen identification were also determined through Chi-square analysis and goodness of fit test. Statistical differences were determined as $P < 0.05$.

Table 1. Overall number of hens positive for *Salmonella* and *Campylobacter* spp. from each commercial housing system at 77 wk of age.

Housing system	*Salmonella* spp. detected no. hens (% total[1])	*Campylobacter* spp. no. hens (% total[1])
Aviary	4 (3.33%)	102 (85.00%)
Conventional cage	16 (13.33%)	114 (95.00%)
Enriched colony cage	6 (5.00%)	109 (90.83%)
P-value	<0.01	<0.05
Column total[2]	26 (7.22%)	325 (90.28%)

[1]Number and percentage of 120 hens within a housing system with at least one positive tissue cultured at 77 wk of age for the selected pathogen.
[2]Number and percentage of the complete set of 360 hens tested across housing systems with at least on positive tissue cultured at 77 wk of age for the selected pathogen.

RESULTS AND DISCUSSION

Prevalence of Salmonella *and* Campylobacter *spp. in Tissues*

The impact of housing system on the prevalence of *Salmonella* and *Campylobacter* detected in at least one tissue of laying hens at flock termination is shown in Table 1. Conventional cage (13.33%) hens had a greater ($P < 0.01$) prevalence of *Salmonella* contamination compared to enriched colony cage (5%) and aviary (3.33%). During an oral challenge of hens in aviary, conventional, and enriched colony cage systems, De Vylder et al. (2009) only found significant differences in *Salmonella* colonization of livers with conventional cage having a greater colonization rate. When comparing floor and two styles of conventional cage production, Green et al. (2009) found a low detection rate of *Salmonella* in intestinal homogenates that was not significant. Across all housing systems in the current study, 7.22% of 360 tested hens had at least one sample positive for *Salmonella*. A high percentage of hens (> 85%) had at least one tissue positive for *Campylobacter* across all housing systems (n = 360 hens). A significantly higher ($P < 0.05$) proportion of conventional hens (95%) were *Campylobacter* positive compared to enriched colony cage (91%) and aviary (85%). Across all housing systems, 90% of hens sampled were contaminated with *Campylobacter*.

The prevalence of *Salmonella* and *Campylobacter* in collected tissues from the housing systems is found in Table 2. Of the 360 spleens assessed across housing systems at flock termination, none were found to contain *Salmonella*. Aviary and conventional cage systems each had a single hen with *Salmonella* detected in the adrenal gland (0.83% of samples from each system; $P > 0.05$). *Salmonella* was found at a significantly higher ($P < 0.0001$) prevalence in conventional cage ceca compared to aviary and enriched colony cage (12.5% vs 2.5%, respectively). Follicles from enriched colony cage hens were contaminated with *Salmonella* at a significantly greater (2.5%; $P < 0.05$) rate, with none detected in the other housing systems.

Campylobacter was recovered at a significantly higher rate from adrenal glands in aviary (14.17%; $P < 0.05$) than from conventional or enriched colony cages (5% each). The spleens of hens across housing systems had a very low prevalence of *Campylobacter* contamination. Approximately 88% of the 360 hens sampled across the three housing systems had *Campylobacter* colonized ceca. A significantly higher occurrence was seen in conventional and enriched colony cage hens (92.5 and 90%, respectively; $P < 0.05$) compared to aviary hens (80.83%). Conversely, a significantly higher percentage of aviary hen follicles (9.17%; $P < 0.05$) were contaminated with *Campylobacter* compared to conventional (4.17%) and enriched colony cage (1.67%). The prevalence of *Campylobacter* in the reproductive tract ranged from 5.83 to 12.5% across the housing systems and was not significantly different ($P > 0.05$). Cox et al. (2012) have reported that *Campylobacter* colonization is not limited to the gastrointestinal tract of poultry, which is also seen in the current study. The rate of *Campylobacter* contamination in various tissues of broiler breeders (Cox et al., 2006) does not correspond to those found in laying hens in various housing systems in that spleens were contaminated at a much lower incidence in the current study and ceca were colonized at a much higher rate than broiler breeders.

Table 2. Impact of commercial housing system on *Salmonella* and *Campylobacter* spp. prevalence in various tissues of laying hens at 77 wk of age.

	Salmonella spp.[1,2]				*Campylobacter* spp.[2]				
Housing system	Adrenal	Ceca	Follicles	Reproductive tract	Adrenal	Spleen	Ceca	Follicles	Reproductive tract
Aviary	1 (0.83%)	3 (2.50%)		1 (0.83%)	17 (14.17%)	2 (1.67%)	97 (80.83%)	11 (9.17%)	15 (12.50%)
Conventional cage	1 (0.83%)	15 (12.50%)			6 (5.00%)		111 (92.50%)	5 (4.17%)	12 (10.00%)
Enriched colony cage		3 (2.50%)	3 (2.50%)		6 (5.00%)	1 (0.83%)	108 (90.00%)	2 (1.67%)	7 (5.83%)
P-value	NS[4]	<0.0001	<0.05	NS	<0.05	NS	<0.05	<0.05	NS
Column total[3]	2 (0.56%)	21 (5.83%)	3 (0.83%)	1 (0.28%)	29 (8.08%)	3 (0.83%)	316 (87.78%)	18 (5.00%)	34 (9.44%)

[1]No *Salmonella* spp. were detected in any spleen samples.
[2]Number and percentage of 120 hens within a housing system positive at 77 wk of age for the selected pathogen within the target tissue.
[3]Number and percentage of the complete set of 360 hens tested across housing systems positive at 77 wk of age for the selected pathogen within the target tissue.
[4]NS = not significant ($P > 0.05$).

Table 3. *Salmonella* spp. identification from environmental swabs of commercial hen housing systems.

Sample type	*Salmonella* Braenderup no. isolates (% total)	*Salmonella* Kentucky no. isolates (% total)	Total no. isolates (% total)
Aviary overall[1] (n = 176)	**26 (23.21%)**	**22 (19.64%)**	**48 (42.86%)**
Aviary drag swabs[2,3]	2 (1.79%)	9 (8.04%)	11 (9.82%)
Aviary nest box	14 (12.50%)	8 (7.14%)	22 (19.64%)
Aviary system wire	10 (8.93%)	5 (4.46%)	15 (13.39%)
Conventional overall (n = 80)	**19 (16.96%)**	**1 (0.89%)**	**20 (17.86%)**
Conventional system wire	19 (16.96%)	1 (0.89%)	20 (17.86%)
Enriched overall (n = 240)	**40 (35.71%)**	**4 (3.57%)**	**44 (39.29%)**
Enriched nest box	13 (11.61%)	ND[4]	13 (11.61%)
Enriched scratch pad	14 (12.50%)	4 (3.57%)	18 (16.07%)
Enriched system wire	13 (11.61%)	ND	13 (11.61%)
Column total	**85 (75.89%)**	**27 (24.11%)**	

[1]Comparison of total *Salmonella* spp. identified within each of the housing systems ($P < 0.0001$).
[2]Percentage of total *Salmonella* spp. identified from each of the environmental sample types ($P < 0.0001$).
[3]Environmental swabs: n = 80 with exception of aviary drag swabs: n = 16.
[4]ND = none detected.

Identification of Detected Salmonella *and* Campylobacter *spp.*

Isolates from Environmental Swabs and Eggshell Pools. The identification of *Salmonella* isolates from environmental swabs collected during the study is presented in Table 3. Throughout the environmental sampling scheme described by Jones et al. (2015), 112 environmental *Salmonella* were detected. Identification procedures determined only two serotypes were isolated: *S.* Braenderup and *S.* Kentucky. Approximately 76% of the environmental isolates were *S.* Braenderup with a significantly greater prevalence (35.71% of all environmental *Salmonella*; $P < 0.0001$, general comparison amongst systems) in the enriched colony cage environment. Overall, the greatest prevalence of *S.* Kentucky was found in the aviary environment (19.64% of all environmental *Salmonella*). When comparing *Salmonella* identification amongst the environmental sample types, *S.* Braenderup was recovered most frequently (16.96% of environmental *Salmonella*) from conventional cage system wires swabs, even though the conventional cage system had the fewest possible environmental swabs due to system design ($P < 0.0001$, comparison between environmental sample types). The fewest number of *S.* Braenderup were found in aviary drag swabs. Amongst environmental sample types, aviary samples more frequently resulted in the detection of *S.* Kentucky. Pieskus et al. (2008) compared fecal, dust, and water samples from aviary, conventional, and enriched colony cage systems and found no difference in *Salmonella* prevalence. *Salmonella* Enteritidis and Typhimurium were the primary isolates detected in the study comparing site visits to several commercial farms in Lithuania. Additionally, during regulatory site visits and sampling of laying farms in Great Britain, Carrique-Mas et al. (2009) found 264 incidents of *Salmonella* detection in fecal and dust samples from 152 laying houses. *Salmonella* Enteritidis (53%) was the primary isolate, while *Salmonella* Mbandaka, Kentucky, and Braenderup were also detected. Additionally, Schulz et al. (2011) screened laying production environments in Belgium, Denmark, and Germany and found *S.* Enteritidis most often and none of the isolates of the serotype were detected in the current study. Rousi et al. (2010) found 64% of the Greek laying hen houses tested were positive for more than one *Salmonella* spp. *Salmonella* Enteritidis was also the primary isolate identified, but *S.* Braenderup was fourth in prominence. Huneau-Salaün et al. (2009) surveyed laying flocks in France and determined the prominence of isolates to be: *S.* Typhimurium, *S.* Enteritidis, *S.* Mbandaka, and *S.* Braenderup.

A significant prevalence ($P < 0.0001$) of *Salmonella* isolates was found in manure scraper blade swabs collected from the three housing systems (Table 4). A greater prevalence of *S.* Braenderup (39.56% of all manure scraper blade isolates) was detected in the conventional cage swabs and of *S.* Kentucky in the enriched colony cage (38.46%). *Salmonella* Braenderup and Kentucky were isolated at a similar prevalence across all the housing systems (46.70 and 52.75%, respectively) although a single isolate of *S.* Mbandaka was detected in the conventional cage system manure scraper swabs. Aviary manure scraper swabs were all positive for *Salmonella* (Jones et al., 2015). Sixty-two percent of the aviary manure scraper swabs were identified as *S.* Kentucky, which was the primary isolate detected in aviary drag swabs. Ninety-one percent of conventional cage manure scraper blade swabs were *S.* Braenderup which was the predominant isolate found in corresponding system wire swabs. Conversely, 98% of enriched colony cage manure scraper blade swabs were identified as *S.* Kentucky, whereas 91% of environmental swabs were *S.* Braenderup. In a multi-national survey of laying hens in the EU, Van Hoorebeke et al. (2010) detected a variety of *Salmonella* isolates, noting patterns of isolate detection within a country.

Only *Salmonella* Braenderup and Kentucky were detected in eggshell pools (Table 5). Of the 393 nest run eggshell pools collected during the study, 22 (across housing systems) contained *Salmonella*. Little et al.

Table 4. *Salmonella* spp. identification from manure scraper blades associated with commercial hen housing systems ($P < 0.0001$).

Sample type	*Salmonella* Braenderup no. isolates (% total[1])	*Salmonella* Kentucky no. isolates (% total)	*Salmonella* Mbandaka no. isolates (% total)	Total no. isolates (% total)
Aviary manure scraper (n = 32)	12 (6.59%)	20 (10.99%)		32 (17.58%)
Conventional manure scraper (n = 80)	72 (39.56%)	6 (3.30%)	1 (0.55%)	79 (43.41%)
Enriched manure scraper (n = 80)	1 (0.55%)	70 (38.46%)		71 (39.01%)
Column total	85 (46.70%)	96 (52.75%)	1 (0.55%)	

[1]Percentage of total *Salmonella* spp. presented in the table.

Table 5. *Salmonella* spp. identification from eggshell pools associated with commercial hen housing systems.

Sample type	*Salmonella* Braenderup no. isolates (% total)	*Salmonella* Kentucky no. isolates (% total)	Total no. isolates (% total)
Aviary overall[1]	**6 (27.27%)**	**4 (18.18%)**	**10 (45.45%)**
Aviary floor[2] (n = 77)	4 (18.18%)	2 (9.09%)	6 (27.27%)
Aviary nest box (n = 80)	ND[3]	1 (4.55%)	1 (4.55%)
Aviary system wire (n = 63)	2 (9.09%)	1 (4.55%)	3 (13.64%)
Conventional overall	**5 (22.73%)**	**1 (4.55%)**	**6 (27.27%)**
Conventional system wire (n = 80)	5 (22.73%)	1 (4.55%)	6 (27.27%)
Enriched overall	**4 (18.18%)**	**2 (9.09%)**	**6 (27.27%)**
Enriched nest box (n = 80)	4 (18.18%)	2 (9.09%)	6 (27.27%)
Enriched system wire (n = 13)	ND	ND	ND
Column total	**15 (68.18%)**	**7 (31.82%)**	

[1]Comparison of total *Salmonella* spp. identified within each of the housing systems ($P > 0.05$).
[2]Percentage of total *Salmonella* spp. identified from each of the eggshell pool sample types ($P > 0.05$).
[3]ND = none detected.

(2008) surveyed eggs in food service in the United Kingdom between 2005 and 2006 and only detected six positive samples (5 – *S.* Enteritidis; 1 – *S.* Mbandaka). Gondek et al. (2013) detected no *Salmonella* on shell surfaces sampled in four housing systems. In the current study, the prevalence of *Salmonella* identification in eggshell pools between commercial housing systems and between the various eggshell pool sample types were not significantly different (Table 5).

Two serotypes of *Campylobacter* were detected throughout the study: *C. coli* and *C. jejuni*. Sahin et al. (2015) report *C. jejuni* as the primary cause of campylobacteriosis, followed by *C. coli*. Table 6 presents the prevalence of each *Campylobacter* isolate in environmental samples collected from the three hen housing systems. A greater amount of *C. jejuni* was identified in the less intensive housing system environments ($P < 0.001$). The aviary and enriched colony cage housing system enrichments present more environmental sampling options than the conventional cage system. As reported in Jones et al. (2015), the scratch pads located in the enriched colony cage system in the current study were a reservoir for *Campylobacter*, primarily *C. jejuni* (19.63% of all environmental *Campylobacter* isolates; 6 samples were positive for both *C. coli* and *C. jejuni*). Environmental *Campylobacter* detected on conventional cage system wire swabs were evenly distributed between *C. coli* and *C. jejuni*.

The frequency of *C. coli* and *jejuni* identification from manure scraper blade swabs is presented in Table 7. *C. coli* was most frequently detected from manure scraper blades, in particular from the aviary (12 of 13 *Campylobacter* positive aviary manure scraper blade swabs; $P < 0.05$). A single *C. jejuni* isolate was found in aviary manure scraper blade swabs which is in opposition to aviary system swabs which contained higher levels *C. jejuni*. Enriched colony cage manure scraper blade swabs had equal frequency of *C. coli* and *C. jejuni*. Enriched colony cage environmental swabs contained significantly ($P < 0.001$) more *C. jejuni* than *C. coli*. Jones et al. (2015) have previously discussed the less than favorable *Campylobacter* growth conditions on the dry manure collection belts in the conventional cage system in particular resulting in no *Campylobacter* being detected in the manure scraper blade swabs.

No significant differences in *C. coli* and *C. jejuni* identification were found for the eggshell pools assessed (Table 8). A total of 297 eggshell pools were assessed for *Campylobacter* spp. identification. Only 12 of the pools were positive with no significant difference ($P > 0.05$) in species identification frequency. Gondek et al. (2013) detected *Campylobacter* on the eggshell surface only in deep litter and free range production systems.

Isolates from Tissues at 77 wk of Age. Twenty-six of the 360 hens sampled across the housing systems at 77 wk of age were positive for *Salmonella* (Table 1). The greatest number of *Salmonella* positive hens was found in the conventional cage system (16 hens, 13.33% of the conventional cage hens examined). All *Salmonella* positive conventional cage hens were contaminated with *Salmonella* Braenderup (15 ceca; 1 adrenal gland) which was also the predominant isolate from conventional cage manure scraper blade swabs. Six enriched colony cage hens (5% of the hens examined in the system) were positive, all identified as *S.* Kentucky

Table 6. *Campylobacter* spp. identification from environmental swabs of commercial hen housing systems.

Sample type	*Campylobacter coli* no. isolates (% total[1])	*Campylobacter jejuni* no. isolates (% total)	Total no. isolates (% total)
Aviary overall[1] (n = 132)	**12 (5.48%)**	**49 (22.37%)**	**61 (27.85%)**
Aviary drag swabs[2,3]	3 (1.37%)	9 (4.11%)	12 (5.48%)
Aviary nest box	1 (0.46%)	4 (1.83%)	5 (2.28%)
Aviary system wire	8 (3.65%)	36 (16.44%)	44 (20.09%)
Conventional overall (n = 60)	**16 (7.31%)**	**15 (6.85%)**	**31 (14.16%)**
Conventional system wire	16 (7.31%)	15 (6.85%)	31 (14.16%)
Enriched overall (n = 180)	**25 (11.42%)**	**102 (46.58%)**	**127 (57.99%)**
Enriched nest box	4 (1.83%)	28 (12.79%)	32 (14.61%)
Enriched scratch pad	18 (8.22%)	43 (19.63%)	61 (27.85%)
Enriched system wire	3 (1.37%)	31 (14.16%)	34 (15.53%)
Column total	**53 (24.20%)**	**166 (75.80%)**	

[1]Comparison of total *Campylobacter* spp. identified within each of the housing systems ($P < 0.001$).
[2]Percentage of total *Campylobacter* spp. identified from each of the environmental sample types ($P < 0.01$).
[3]Environmental swabs: n = 60 with exception of aviary drag swabs: n = 12.

Table 7. *Campylobacter* spp. identification from manure scraper blades associated with commercial hen housing systems ($P < 0.05$).

Sample type	*Campylobacter coli* no. isolates (% total[1])	*Campylobacter jejuni* no. isolates (% total)	Total no. isolates (% total)
Aviary manure scraper (n = 24)	12 (26.67%)	1 (2.22%)	13 (28.89%)
Conventional manure scraper (n = 60)	ND[2]	ND	ND
Enriched manure scraper (n = 60)	17 (37.78%)	15 (33.33%)	32 (71.11%)
Column total	29 (64.44%)	16 (35.56%)	

[1]Percentage of total *Campylobacter* spp. presented in the table.
[2]ND = none detected.

Table 8. *Campylobacter* spp. identification from eggshell pools associated with commercial hen housing systems.

Sample type	*Campylobacter coli* no. isolates (% total)	*Campylobacter jejuni* no. isolates (% total)	Total no. isolates (% total)
Aviary overall[1]	**1 (8.33%)**	**5 (41.67%)**	**6 (50.00%)**
Aviary floor[2] (n = 57)	ND[3]	1 (8.33%)	1 (8.33%)
Aviary nest box (n = 60)	1 (8.33%)	1 (8.33%)	2 (16.67%)
Aviary system wire (n = 49)	ND	3 (25.00%)	3 (25.00%)
Conventional overall		**1 (8.33%)**	**1 (8.33%)**
Conventional system wire (n = 60)	ND	1 (8.33%)	1 (8.33%)
Enriched overall	**1 (8.33%)**	**4 (33.33%)**	**5 (41.67%)**
Enriched nest box (n = 60)	1 (8.33%)	3 (25.00%)	4 (33.33%)
Enriched system wire (n = 11)	ND	1 (8.33%)	1 (8.33%)
Column total	**2 (16.67%)**	**10 (83.33%)**	

[1]Comparison of total *Campylobacter* spp. identified within each of the housing systems ($P > 0.05$).
[2]Percentage of total *Campylobacter* spp. identified from each of the eggshell pool sample types ($P > 0.05$).
[3]ND = none detected.

(the predominant isolate from enriched colony cage manure scraper blade swabs). Three hens had positive ceca and three hens had contaminated follicles. Four aviary hens were positive for *Salmonella* (3.33% of the aviary hens examined), although 5 tissue samples were contaminated (one hen had positive ceca and reproductive tract). Three ceca and an adrenal gland were contaminated with *S.* Kentucky, which again, was the primary isolate from aviary manure scraper blade swabs. The single positive reproductive tract from the study was from an aviary hen which had 2 sampled tissues contaminated. The reproductive tract was contaminated with *S.* Cubana.

At 77 wk of age, tissues collected across the housing systems were contaminated at an equal rate with *Campylobacter coli* and *C. jejuni* (Table 9). Many hens experienced multiple tissue *Campylobacter* contaminations (aviary = 32; conventional = 23; enriched colony = 17). *C. coli* was isolated at a significantly ($P < 0.0001$) higher rate from tissues of aviary and conventional cage hens (20.05 and 20.77% of total tissue isolates, respectively), whereas *C. jejuni* was significantly more prominent in enriched colony cage hens (21.74% of total tissue isolates). Enriched colony cage hens had 15 fewer *Campylobacter* isolates detected in tissues at 77 wk of age compared to aviary and conventional cage hens.

The distribution of *Campylobacter* isolates identified by tissue and housing system is presented in Table 10. There was no difference in the prevalence of *C. coli*

Table 9. Overall identification of *Campylobacter* spp. in tissues from each commercial housing system at 77 wk of age ($P < 0.0001$).

Sample type	*Campylobacter coli* no. isolates (% total[1])	*Campylobacter jejuni* no. isolates (% total)	Total no. isolates (% total)
Aviary	83 (20.05%)	60 (14.49%)	143 (34.54%)
Conventional cage	86 (20.77%)	57 (13.77%)	143 (34.54%)
Enriched colony cage	38 (9.18%)	90 (21.74%)	128 (30.92%)
Column total	207 (50.00%)	207 (50.00%)	

[1]Percentage of total *Campylobacter* spp. presented in the table.

Table 10. Impact of commercial housing system on *Campylobacter* spp. identification in various tissues of laying hens at 77 wk of age.[1]

Housing system	Adrenal		Spleen		Ceca		Follicles		Reproductive tract	
	C. coli	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*
Aviary	6 (20.69%)	11 (37.93%)	1 (33.33%)	1 (33.33%)	60 (18.29%)	38 (11.59%)	5 (26.32%)	6 (31.58%)	11 (31.43%)	4 (11.43%)
Conventional cage	3 (10.34%)	3 (10.34%)	ND[2]	ND	75 (22.87%)	44 (13.41%)	1 (5.26%)	5 (26.32%)	7 (20.00%)	5 (14.29%)
Enriched colony cage	1 (3.45%)	5 (17.24%)	ND	1 (33.33%)	34 (10.37%)	77 (23.48%)	ND	2 (10.53%)	3 (8.57%)	5 (14.29%)
Column total	10 (34.48%)	19 (65.52%)	1 (33.33%)	2 (66.67%)	169 (51.52%)	159 (48.48%)	6 (31.58%)	13 (68.42%)	21 (60.00%)	14 (40.00%)
P-value	NS		NS		< 0.0001		NS		NS	

[1]Number of isolates detected and percentage of *Campylobacter* spp. detected within the target tissue.
[2]ND = none detected.

and *C. jejuni* contamination of adrenal glands amongst housing systems. Very few spleens were contaminated with *Campylobacter* and results were not significantly different between the housing systems. Ceca were highly colonized with both *C. coli* and *C. jejuni*. A significantly higher percentage of aviary and conventional cage ceca were colonized with *C. coli* (18.29 and 22.87% of ceca isolates, respectively), whereas *C. jejuni* was significantly greater in enriched colony cage (23.48%). Enriched colony cage environmental swabs were also predominantly contaminated with *C. jejuni*, but so were aviary environmental swabs and conventional cage swabs were equally contaminated with the two isolates. A small number of hens across all the housing systems had follicles contaminated with *Campylobacter* and there was no significant difference in the prevalence of *C. coli* and *C. jejuni*. Reproductive tract contamination with *C. coli* and *C. jejuni* occurred across all housing systems at a low (less than 10% of hens tested within a housing system), non-significant rate.

According to the Center for Disease Control and Prevention FoodNet data (Crim et al., 2014), in 2013 there were 19,056 human cases of culture-confirmed pathogen infections in the US. Of these, 7,277 were *Salmonella* and 6,621 were *Campylobacter*; followed by 2,309 cases of *Shigella*. The poultry production environment is a favorable reservoir for both *Salmonella* and *Campylobacter*. Comparing commercial hen housing systems has determined that *Salmonella* and *Campylobacter* frequently are detected, but nest run eggshell pools are infrequently contaminated.

Considering the diversity of known *Salmonella* spp., the results of the current study detecting a predominance of *Salmonella* Braenderup and *S.* Kentucky (with single isolate detection of each *S.* Mbandaka and *S.* Cubana) are surprising. The primary laboratory confirmed isolate for *Salmonella* infection in the US in 2012 was *S.* Enteritidis, followed by *S.* Typhimurium and *S.* Newport (CDC, 2014). *Salmonella* Braenderup was ranked eleventh accounting for 1.7% of human infections. Further in the report, the primary laboratory-confirmed, non-clinical non-human source submitted to the National Veterinary Services Laboratory was *Salmonella* Kentucky, followed by *S.* Enteritidis and *S.* Heidelberg. *Salmonella* Braenderup ranked tenth on the list. Most researchers report a high prevalence of *S* Enteritidis, *S.* Typhimurium, and *S.* Heidelberg in egg production environments. That was not found in the current comparison of commercial cage-free aviary, conventional cage, and enriched colony cage housing systems. While *S.* Braenderup and *S.* Kentucky are capable of human pathogenicity, they are not often associated with foodborne outbreaks. The frequency of *S.* Kentucky detection in the animal agriculture environment (CDC, 2014) appears to be on the increase and leads to new questions as to why this might be occurring. The current study has identified housing system design and management areas (enriched colony cage scratch pads, aviary floor eggs) to assess for enhancing hen health and product safety.

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