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Horizontal Transmission of *Salmonella* and *Campylobacter* Among Caged and Cage-Free Laying Hens

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SUMMARY. In each of five sequential trials, laying hens (56–72 wk of age) were challenged with *Salmonella* and *Campylobacter*, and 1 wk postinoculation, the challenged hens ($n = 3$) were commingled with nonchallenged hens ($n = 12$) in conventional wire cages, on all-wire slats, or on all-shavings floor housing systems. After 12 days, challenged and nonchallenged hens were euthanatized for sample collection. Ceca were aseptically collected from all hens, and the spleen, liver/gallbladder (LGB), lower (LRT) and upper (URT) reproductive tracts, and ovarian follicles (mature and immature) were collected from only the challenged hens after commingling. Samples were divided equally and cultured separately for *Salmonella* and *Campylobacter*. Differences in the horizontal transmission of the challenge *Salmonella* to nonchallenged hens housed in cages (12%), on slats (15%), and on shavings (14%) were not significantly different ($P > 0.05$) from the challenged pen-mate hens over the five trials. However, with the inclusion of residual environmental *Salmonella*, the recovery of *Salmonella* from nonchallenged hens housed in cages was lowest at 15%, intermediate for hens on slats at 20%, and highest for hens on shavings at 38%. Among challenged hens housed in cages, *Salmonella* was recovered from only 27% of the cecum and LRT samples. From challenged hens housed on slats, *Salmonella* was recovered from 38% of the cecum, 12% of the spleen, 19% of the LGB, 44% of the LRT, and 19% of the URT samples. From challenged hens housed on shavings, *Salmonella* was recovered from 31% of the cecum; 15% of the spleen, LGB, and URT; and 31% of the LRT samples. Horizontal transmission of *Campylobacter* among nonchallenged pen-mate hens was significantly lower for hens housed in cages at 28% than for hens on shavings at 47%, with hens on slats being intermediate at 36%. For challenged hens housed in cages, *Campylobacter* was recovered from 27% of the cecum, 13% of the LRT, 7% of the URT, and 17% of the follicle samples. Among the challenged hens housed on slats, *Campylobacter* was recovered from 44% of the cecum, 6% of the spleen, 19% of the LGB, 12% of the LRT, 6% of the URT, and 14% of the follicle samples. Among challenged hens housed on shavings, *Campylobacter* was recovered from 46% of the cecum, 8% of the LRT and URT, and 40% of the follicle samples. The overall results of this study indicate that the caged housing system provided the lowest horizontal transmission level of *Salmonella* and *Campylobacter* among egg-laying hens.

RESUMEN. La transmisión horizontal de *Salmonella* y *Campylobacter*, entre gallinas ponedoras en jaula y no enjauladas.

En cada uno de los cinco ensayos secuenciales, se desafiaron gallinas de postura (56–72 semanas de edad) con *Salmonella* y *Campylobacter*, y una semana después de la inoculación, las gallinas desafiadas ($n = 3$) fueron mezcladas con gallinas no desafiadas ($n = 12$) y se alojaron en jaulas de alambre convencionales jaulas, en piso de rejilla de alambre en piso con virutas de madera. Doce días después, se practicó la eutanasia a las gallinas desafiadas y no desafiadas para recolectar muestras. Los sacos ciegos se recolectaron de forma aséptica de todas las gallinas. También se recolectaron el bazo, el hígado con la vesícula biliar, los tractos reproductivos inferiores (LRT) y superiores (URT), y los folículos ováricos (maduros e inmaduros) solamente de las gallinas que fueron desafiadas después del contacto. Las muestras se dividieron en partes iguales y se cultivaron por separado para *Salmonella* y *Campylobacter*. No se observaron diferencias significativas ($P > 0.05$) en la transmisión horizontal de *Salmonella* a las gallinas no desafiadas entre las gallinas alojadas en jaulas (12%), en piso de rejillas (15%), y en viruta de madera (14%) en comparación no fueron significativamente en relación con las aves desafiadas en los cinco ensayos. Sin embargo, con la inclusión de *Salmonella* ambiental residual, la menor recuperación de *Salmonella* de gallinas alojadas en jaulas sin desafiar con un 15%, intermedia para las gallinas en piso de rejillas con un 20% y la más alta fue para gallinas en virutas con un 38%. Se recuperó *Salmonella* de las gallinas desafiadas alojadas en jaulas solamente en el 27% de las muestras de ciego y del tracto reproductor inferior. De las gallinas desafiadas alojadas en piso de rejillas, se recuperó *Salmonella* en un 38% de los ciegos, en 12% de los bazos, en 19% del hígado y vesícula biliar, en un 44% del tracto reproductor inferior, y en el 19% de las muestras del tracto reproductor superior. De gallinas desafiadas alojadas en piso con viruta de madera, se recuperó *Salmonella* en un 31% del ciego, en 15% del bazo, del hígado y vesícula biliar y del tracto reproductor superior, y por último en el 31% de las muestras del tracto reproductor inferior. La transmisión horizontal de *Campylobacter* entre las gallinas no desafiadas fue significativamente menor para las gallinas alojadas en jaulas con un 28% en comparación con las gallinas en viruta de madera con un 47%, con las gallinas en piso de rejillas resultó ser intermedia con un 36%. Para las gallinas desafiadas alojadas en jaulas desafió, se recuperó *Campylobacter* en un 27% de los ciego, en 13% del tracto reproductor superior, en 7% del tracto reproductor superior y en 17% de las muestras de folículos. Entre las gallinas desafiadas y alojadas en rejillas, se recuperó *Campylobacter* de un 44% de los ciegos, del 6% de los bazos, del 19% del hígado y vesícula biliar, del 12% del tracto reproductor inferior, del 6% del tracto reproductor superior y del 14% de las muestras de folículos. Entre las gallinas desafiadas ubicadas en viruta de madera, se recuperó *Campylobacter* de un 46% de los ciegos, del 8% de los tractos reproductores inferior y superior y del 40% de las muestras de folículos. Los resultados globales de este estudio indican que el sistema de alojamiento en jaulas proporcionó siempre el nivel más bajo de transmisión horizontal de *Salmonella* y *Campylobacter* en las gallinas ponedoras.

Key words: *Salmonella*, *Campylobacter*, horizontal transmission, cage-free laying hens, caged laying hens

Abbreviations: BGS = Brilliant Green Sulfa; BPW = buffered peptone water; cfu = colony-forming units; LGB = liver/gallbladder; LRT = lower reproductive tract; URT = upper reproductive tract

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Salmonella and *Campylobacter* are common causes of foodborne bacterial gastroenteritis in the United States and worldwide and are considered to be the most important zoonotic pathogens with regard to poultry (27,32). *Salmonella* infection among laying hens is a primary food safety concern for the commercial table egg industry (21,34) because *Salmonella enterica* serovar Enteritidis is the primary pathogen that intermittently contaminates eggs (26) and eggs are the main food source for transmission of *Salmonella* Enteritidis to humans. Greig and Ravel (25) recently analyzed the international foodborne outbreak data reported between 1988 and 2007 and found that 43% of *Salmonella* Enteritidis outbreaks were associated with eggs. Eggs can become contaminated before oviposition as a result of the reproductive tissues (ovary and oviduct) being infected, at oviposition when the eggshell passes through the cloaca, or after oviposition when the egg comes in contact with contaminated environmental surfaces (20), and these routes of contamination have been identified as transovarian, oviductal, and transeggshell (3,4). The prevalence of *Salmonella* Enteritidis among eggs produced by naturally infected hens is low. Humphrey *et al.* (28) and Poppe *et al.* (39) estimated the overall *Salmonella* Enteritidis prevalence among egg contents as 0.55% and <0.06%, respectively. Using available data on the occurrence of *Salmonella* Enteritidis in U.S. laying hens and eggs, Ebel and Schlosser (18) predicted that one in every 20,000 (0.005%) eggs produced annually could be contaminated with *Salmonella* Enteritidis.

Although *Campylobacter* has been recovered from the reproductive tissues of broiler breeder (5,9) and laying hens (8,11), eggs have not been identified as a significant foodborne source of *Campylobacter* infection in humans. Studies have shown that the prevalence of *Campylobacter* among table eggs is low (1,30,44). *Campylobacter* spp. accounted for 0.6% of international egg-associated foodborne outbreaks, whereas *Salmonella* spp. accounted for 97.4% (25). When sampling eggs produced by laying hens determined to be fecally positive for *Campylobacter jejuni*, Doyle (15) recovered *C. jejuni* from only two of 226 eggshell surfaces and no egg contents. *Campylobacter jejuni* was also recovered from the interior eggshell and shell membranes of inoculated eggs subjected to refrigeration at 4 C for up to 72 hr (15). Neill *et al.* (35) recovered *C. jejuni* from shell membranes, but not from the albumen or yolk after immersion challenge and incubation at 37 C for 1, 3, or 6 hr, with no recovery after 24 hr.

In the national and international table egg industries, commercial laying hens are primarily housed in conventional battery cages (2,27). Although there are several advantages to cage management, including lower production costs, increased egg production, and increased hen livability, this housing system has been extensively criticized for providing a barren and confined environment that physically restricts laying hens from performing many of their natural behaviors (38,42,45). These concerns have led to the development and proposal of legislation in the United States and other countries to phase out conventional cages and implement alternative systems intended to improve hen welfare (7,17,33,42). Conventional cages will also be banned in the European Union by 2012 (19). To address growing hen welfare concerns associated with caged housing and to meet consumer demand for cage-free products, a portion of table egg producers have transitioned to alternative, cage-free production systems (24). It has been estimated that 5% of U.S. table eggs in 2009 were produced by hens housed in alternative production systems (46).

Several studies have focused on the effects of housing system on eggshell contamination of table eggs (13,31,40), but few studies have evaluated the effects of housing system on the prevalence and transmission of *Salmonella* and *Campylobacter* among laying hens housed in cage and cage-free systems. Pieskus *et al.* (37) showed no significant difference in the prevalence of *Salmonella* between hens housed in cage and aviary systems. Green *et al.* (24) found no differences in *Salmonella* prevalence between caged and cage-free laying hens, and De Vylder *et al.* (14) concluded that housing laying hens in alternative production systems would not increase *Salmonella* colonization or shedding. One of the main advantages associated with conventional cages is that, because hens are efficiently separated from their feces, the risk for fecally shed and transmitted diseases among hens is reduced. However, with flock size being typically larger in caged housing systems (5- to 15-fold) than in cage-free housing systems, Van Hoorebeke *et al.* (47) considered housing laying hens in conventional cages a significant risk factor for the spread of *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Campylobacter* has been recovered from naturally infected caged (11) and cage-free (44) laying hens, but limited data is available on the influence of these production systems on *Campylobacter* transmission among laying hens. Housing environment has been reported to play an important role in the recovery of *C. jejuni* from broiler chickens, with *C. jejuni* being recovered from floor-raised (65%) broilers at a significantly higher level than cage-raised (37%) broilers (48). As some table egg producers change to alternative and cage-free systems to comply with legislative requirements and to meet consumer demand, it is important to determine what effect housing systems could have on the spread of *Salmonella* and *Campylobacter* among laying hens housed on the floor.

The objectives of this study were to compare the horizontal transmission and colonization ability of *Salmonella* Typhimurium and *Salmonella* Enteritidis marker strains in conjunction with *Campylobacter coli* and *C. jejuni* strains in conventional wire cages, on all-wire slats, or on all-shavings floor housing systems for laying hens.

MATERIALS AND METHODS

Inoculation and experimental design. In each of five sequential trials, nine laying hens (from a single flock of Hy-Line W-36 white and Hy-Line brown at 56, 61, 65, 70, and 72 wk of age) were challenged by three routes: orally (1 ml), intravaginally (1 ml), and intracolonically (1 ml) with *Salmonella* (day 1) and *Campylobacter* (day 2). A nalidixic acid-resistant marker strain of *Salmonella* Typhimurium (6,12) was used in trials 1 and 2 (average titer, 1.1×10^9 colony-forming units cfu/ml), whereas nalidixic acid–resistant *Salmonella* Enteritidis (both provided by N. A. Cox, USDA/ARS Russell Research Center, Athens, GA) was used in trials 3, 4, and 5 (average level, 3.3×10^8 cfu/ml). A gentamicin-resistant marker strain of *C. coli* (10) was used in trials 1, 2, and 3 (average level 5.9×10^8 cfu/ml), and a field strain of *C. jejuni* (both provided by N. A. Cox) was used in trials 4 and 5 (average level, 1.2×10^8 cfu/ml). Challenged hens were housed in individual wire cages in isolation for 7 days postinoculation to permit intestinal passage of the challenge bacteria load and to establish colonization. During each trial, approximately 5 g of fresh feces was aseptically collected from each challenged hen 4 days postinoculation. Each sample was placed in a sterile 50-ml centrifuge tube and transferred to the laboratory for analysis. A standard volume of 30 ml of buffered peptone water (BPW; 1%; Acumedia, Lansing, MI) was added to each fecal sample, and all samples were vortexed. For *Campylobacter* analysis, 5 ml of the suspension was transferred from each sample to 45 ml of prepared TECRA enrichment broth (Frenchs Forest, NSW, Australia) with supplements (*Campylobacter* selective supplement containing trimethoprim, rifampicin, and polymyxin) and 5 ml into 45 ml of BPW for *Salmonella* analysis. Samples in TECRA broth were incubated microaerobically at 42 C for 48 hr, and samples in BPW were incubated at 37 C for 24 hr.

After 7 days, the caged, challenged hens served as vectors and were commingled with caged, nonchallenged hens at a ratio of one challenged hen per four nonchallenged hens in adjacent pens containing conventional colony cages (0.6 ft^2/hen [0.06 m^2/hen]), all-wire slats (6.4 ft^2/hen [0.6 m^2/hen]), or all-shavings flooring systems (6.4 ft^2/hen [0.6 m^2/hen]). Before hen placement, the pens used in this study were sampled via stepped-on drag swabs (6), and all pens tested negative for *Salmonella* and *Campylobacter*, although the pens had not been cleaned from previous flock use from 15–52 wk of age. The room used for this study contained a duplicate set of pens for each housing system, with a pen for cages, slats, and shavings on each side of the room separated by an access alley. Trials 1, 3, and 5 were conducted in the left set of pens approximately 5 wk apart, whereas trials 2 and 4 were conducted in the right set of pens approximately 6 wk apart. A trough feeder was used for hens in cages, whereas one tube/pan feeder (41.5-inch [105-cm] circumference with 14 partitions) was used for hens housed in the wire slats and shavings pens. A one-story front roll-out nest box with rubber finger nest pads was provided for hens housed on wire slats and shavings at a stocking density of 2.5 hens/nest. Perches (19.1 inches [48.5 cm]/hen) were available in the wire slats and shavings pens. For each trial, a total of 15 hens were placed in each housing system. Commingled hens had access to the same feeding and watering systems, and all hens were subjected to the same room environmental conditions (temperature and humidity ranges, ventilation, light intensity, and photoperiod program).

Environmental samples. In each trial, on the 10th day after commingling, pens were sampled via stepped-on drag swabs ($n = 2$ samples/pen) for *Salmonella* and *Campylobacter*. Presoaked drag swabs (DS-001, Solar Biologicals, Inc., Ogdensburg, NY) were unwound and dragged across the litter beneath the cages, on the slats floor, and on the shavings floor in a figure eight pattern around the pen (6). Swabs were stepped on four times during sampling with a clean, disposable boot cover that was put on when entering each pen. The nipples on each drinker line ($n = 1$ nipple/pen) were also sampled during trials 4 and 5. Using a gloved hand, each nipple ($n = 2$ nipples/cage; $n = 10$ nipples/line in the shavings and slats pens) was sampled with an open gauze swab ($n = 2$ samples/pen). Individual litter and nipple drinker swab samples were placed in a sterile sample bag and transported to the laboratory. One hundred milliliters of BPW were added to each sample. All samples were massaged by hand to loosen any attached debris. Five milliliters of BPW was transferred from each sample to 45 ml of prepared TECRA for *Campylobacter* analysis. Swabs samples in 45 ml of BPW were incubated for 24 hr at 37 C for *Salmonella* analysis. Swab samples in TECRA for *Campylobacter* analysis were incubated microaerobically at 42 C for 48 hr.

Egg samples. For trials 4 and 5, eggs produced by challenged hens during isolation and after commingling were sampled for *Salmonella*. For identification purposes only, brown eggshell–laying hens were challenged, and all nonchallenged hens laid white-shelled eggs. This was done to ensure that after the challenged hens were commingled with nonchallenged hens, brown eggs produced by the challenged hens were easily identified and collected for sampling. Eggs were collected daily, placed on a clean flat, and held in an onsite egg cooler at 12 C and 70% relative humidity until sampled. Eggs from trial 4 were taken to the laboratory for sampling 16 days after collection began and eggs from trial 5 were taken 10 days after collection began. Eggs produced by the challenged hens while in cages before commingling were pooled by hen (trial 4, 3–5 eggs/hen for nine hens; trial 5, 2–5 eggs/hen for nine hens). A total of 42 eggs were collected for sampling in trial 4, and 40 eggs were collected for sampling in trial 5. Eggs produced by the challenged hens after commingling were pooled for each housing system (4–5 eggs/housing system resulting in 21 pools for trial 4 and 11 pools for trial 5). A total of 97 and 54 eggs were collected for sampling in trials 4 and 5, respectively.

In the lab, eggs were cracked on a sterile surface. The internal contents of eggs pooled by hen (produced in isolation) were released into a gloved hand to separate the yolk from the albumen. The albumen was discarded and the yolk transferred to a sterile petri dish. Five milliliters of yolk was collected with a sterile syringe and transferred to a sterile sample bag. The vitelline membrane was then removed from the yolk with sterile forceps, rinsed with distilled water, and transferred to a sterile 50-ml centrifuge tube. The eggshell and adhering membrane complex were crushed by hand and placed in a sterile sample bag. The eggshell and adhering membrane complex, the 5-ml yolk samples, and the vitelline membranes from eggs within each pooled sample were combined by sample type into single sample containers. To maintain an aseptic technique, new gloves and sterile forceps and syringes were used between pooled samples. Eggs collected after hens were commingled were cracked on a sterile surface, the internal contents discarded, and the eggshells and adhering membranes pooled by housing system (for trial 4, $n = 21$ pools; for trial 5, $n = 11$ pools).

Buffered peptone was added to each sample bag at a ratio of 20 ml/eggshell or pooled vitelline membrane and for 10 ml/5 ml of yolk material. The vitelline membrane samples were vortexed, and all samples were incubated for 24 hr at 37 C for *Salmonella* analysis.

Organ samples. Twelve days after commingling, challenged and nonchallenged hens were euthanized by electrocution and samples were collected. Ceca were aseptically collected from both challenged and nonchallenged hens. One cecum was used for *Salmonella* analysis, whereas the other cecum was used for *Campylobacter* analysis. From only the challenged hens, the spleen, liver/gallbladder (LGB), upper (URT: infundibulum, magnum, and isthmus) and lower (LRT: shell gland and vagina) reproductive tract segments and ovarian follicles (mature and immature; trials 4 and 5, only) were collected aseptically. After separating the LRT and URT, each segment was placed on a clean surface and aseptically divided longitudinally, providing one half for *Salmonella* analysis and one half for *Campylobacter* analysis. Each sample was transferred to a sterile sample bag, placed on ice, and transported to the laboratory for analysis. An average weight for each sample type was obtained. The samples within the plastic bags were then macerated with a rubber mallet to expose the internal contents. Physiologic saline (0.85%) was added to the LGB, spleen, and follicle samples at a ratio of one times the weight of the sample (ml/g). All LGB, spleen, and follicle samples were equally divided for *Salmonella* and *Campylobacter* analysis. BPW and TECRA enrichment broth were added at a ratio of 3 ml/g of sample for *Salmonella* and *Campylobacter* analysis, respectively. All samples were then placed in a Stomacher 400 (Fisher Scientific, Hampton, NH) and stomached for 1 min. Samples for *Salmonella* analysis were incubated at 37 C for 24 hr, and samples for *Campylobacter* analysis were incubated microaerobically at 42 C for 48 hr.

Plating procedures. Two loops (20 μl) from each sample for *Salmonella* analysis were streaked onto Brilliant Green Sulfa (BGS) agar containing 200 ppm nalidixic acid. BGS plates were incubated at 37 C for 24 hr, and cfus characteristic of *Salmonella* were selected and subjected to slide agglutination tests using *Salmonella* O antisera (Becton Dickinson, Sparks, MD) for serogroup (trials 1 and 2, group B for Typhimurium; trials 3–5, group D_1 for Enteritidis) confirmation. Samples for *Campylobacter* analysis were streaked (20 μl) onto Campy-Cefex with gentamicin (Sigma Aldrich, St. Louis, MO; trials 1–3, *C. coli*) or without gentamicin (trials 4 and 5, *C. jejuni*). Samples from trials 4 and 5 were also streaked onto Campy-Cefex plates with gentamicin to ensure that any *Campylobacter* recovered was *C. jejuni* and not residual *C. coli*. Campy-Cefex plates were incubated in a microaerobic atmosphere at 42 C for 48 hr. Following incubation, characteristic cfus were confirmed by observation, through phase-contrast microscopy, of the distinctive spiral morphology and darting motility of *Campylobacter* on a wet mount.

Statistical analysis. Chi-square and Fisher exact test (SAS Institute, Inc., Cary, NC) were used to identify differences in *Salmonella* (*Salmonella* Typhimurium or *Salmonella* Enteritidis) and *Campylobacter* (*C. coli* or *C. jejuni*) colonization prevalence due to housing system (cages, slats, or shavings). Differences were considered significant at $P < 0.05$. Fecal and egg sample numbers ($n = 1$–5) were insufficient and therefore were not statistically analyzed.

RESULTS

In trials 1 and 2, before commingling of the hens, *Salmonella* Typhimurium was recovered from 100% (17/17; one hen died) of the

Table 1. Percentage of tissue samples positive for challenge *Salmonella* (*Salmonella* Typhimurium or *Salmonella* Enteritidis) or *Campylobacter* (*C. coli* or *C. jejuni*) from nonchallenged and challenged laying hens within cage, slat, and shaving housing systems (trials 1–5).[A]

Housing system	Nonchallenged hens		Challenged hens						
	n	Cecum[B]	*n*	Cecum	Spleen	LGB	LRT	URT	Follicles[C]
Salmonella (challenge)									
Cages	60	12	15	20	0	0	13	0	0
Slats[D]	61	15	16	25	12	19	25[E]	19	0
Shavings[F]	58	14	13	15	15	8	15	8	0
Salmonella (challenge and environmental)[G]									
Cages	60	15[a]	15	27	0[H]	0[H]	27	0[H]	0[H]
Slats	61	20[a]	16	38	12[H]	19[H]	44	19[H]	0[H]
Shavings	58	38[b]	13	31	15[H]	15	31	15	0[H]
Campylobacter									
Cages	60	28[a]	15	27	0	0	13	7	17
Slats	61	36[ab]	16	44	6	19	12	6	14
Shavings	58	47[b]	13	46	0	0	8	8	40

[A]LGB = liver/gallbladder; LRT = lower reproductive tract (shell gland and vagina); URT = upper reproductive tract (infundibulum, magnum, and isthmus).
[B]Percentages within columns and bacteria type with different lowercase superscripts differ significantly ($P < 0.05$).
[C]Follicles (mature and immature ovarian) sampled only during trials 4 and 5; cages, $n = 6$; slats, $n = 7$; shavings, $n = 5$.
[D]For nonchallenged hens, $n = 61$, and for challenged hens, $n = 16$ because one additional nonchallenged and challenged hen were placed during trial 4.
[E]One of the *Salmonella*-positive LRT samples was the only sample from which *Salmonella* Enteritidis was recovered.
[F]For nonchallenged hens, $n = 58$ because two hens died during trial 5; for challenged hens, $n = 13$ because one hen died during both trials 2 and 5.
[G]Percentages include residual environmental pen *Salmonella* Typhimurium from challenges in trials 1 and 2.
[H]No change; results are the same as reported for challenge *Salmonella*.

fecal samples collected from challenged hens. In trials 3, 4, and 5, before commingling of hens, *Salmonella* Enteritidis was recovered from only 57% (16/28) of the fecal samples collected. *Campylobacter coli* was recovered from 65% (17/26) of the fecal samples collected from challenged hens in trials 1, 2, and 3, and *C. jejuni* was recovered from 100% (19/19) of the fecal samples collected in trials 4 and 5. All eggshell, vitelline membrane, and yolk samples ($n = 10$) from eggs produced by challenged hens held in isolation during trial 4 were negative for *Salmonella* Enteritidis and eggs produced by isolated challenged hens in trial 5, only 1/10 eggshell samples were positive for *Salmonella* Enteritidis, and all other samples were negative.

There was no significant ($P > 0.05$) difference in the horizontal transmission of *Salmonella* (Table 1) among nonchallenged hens housed in cages (12%), on slats (15%), and on shavings (14%). Of the samples collected from challenged hens housed in cages, *Salmonella* was recovered only from the cecum (20%) and LRT (13%). Among challenged hens housed on slats, *Salmonella* was recovered from 25% of the cecum, 12% of the spleen, 19% of the LGB, 25% of the LRT, and 19% of the URT samples. Among challenged hens housed on shavings, *Salmonella* was recovered from 15% of the cecum, spleen, and LRT samples and 8% of the LGB and URT samples. *Salmonella* was not recovered from any of the ovarian follicles sampled. Collectively, *Salmonella* was recovered from 53 tissue organ samples, and approximately 98% of the *Salmonella* recovered was confirmed as *Salmonella* Typhimurium. The only *Salmonella*-positive sample confirmed as *Salmonella* Enteritidis was a LRT collected from a challenged hen housed on slats in trial 3.

Challenged hens were inoculated with *Salmonella* Enteritidis in trials 3, 4, and 5, but *Salmonella* Enteritidis was not recovered from any of the nonchallenged hens used in these three trials. However, residual *Salmonella* Typhimurium from trials 1 and 2 was recovered from cecum samples from nonchallenged hens in these three trials, and when these data (Table 1) were included, the horizontal transmission of *Salmonella* was significantly less in cages (15%) and on slats (20%) than on shavings (38%). Residual *Salmonella* Typhimurium was also recovered from the cecum and LRT of challenged hens in these three trials. Among challenged hens housed in cages, on slats, and on shavings, the percentage of cecum samples positive for *Salmonella* increased to 27%, 38%, and 31%, respectively, and the percentage of LRT samples positive for *Salmonella* increased to 27%, 44%, and 31%, respectively. The only LGB and URT samples positive for residual *Salmonella* Typhimurium were collected from a single hen housed on shavings. All spleen and ovarian follicle samples collected from challenged hens in the last three trials were negative for *Salmonella* Typhimurium.

Horizontal transmission of *Campylobacter* (Table 1) was significantly lower in cages (28%) than on shavings (47%), whereas horizontal transmission on slats (36%) was intermediate to that of the caged and shavings housing systems. Among challenged hens housed in cages, *Campylobacter* was recovered from 27% of the cecum, 13% of the LRT, 7% of the URT, and 17% of the follicle samples. For challenged hens housed on slats, *Campylobacter* was recovered from 44% of the cecum, 6% of the spleen, 19% of the LGB, 12% of the LRT, 6% of the URT, and 14% of the follicle samples. From challenged hens housed on shavings, *Campylobacter* was recovered from 46% of the cecum, 8% of the LRT, 8% of the URT, and 40% of the follicle samples collected. *Campylobacter jejuni* (81/99 positive samples) was recovered from more tissue organ samples than *C. coli* (18/99 positive samples). The percentage of cecum samples positive for *C. coli* and *C. jejuni* is presented in Fig. 1. For both the nonchallenged and challenged hens, only *C. jejuni* was recovered from the caged housing system, whereas *C. coli*

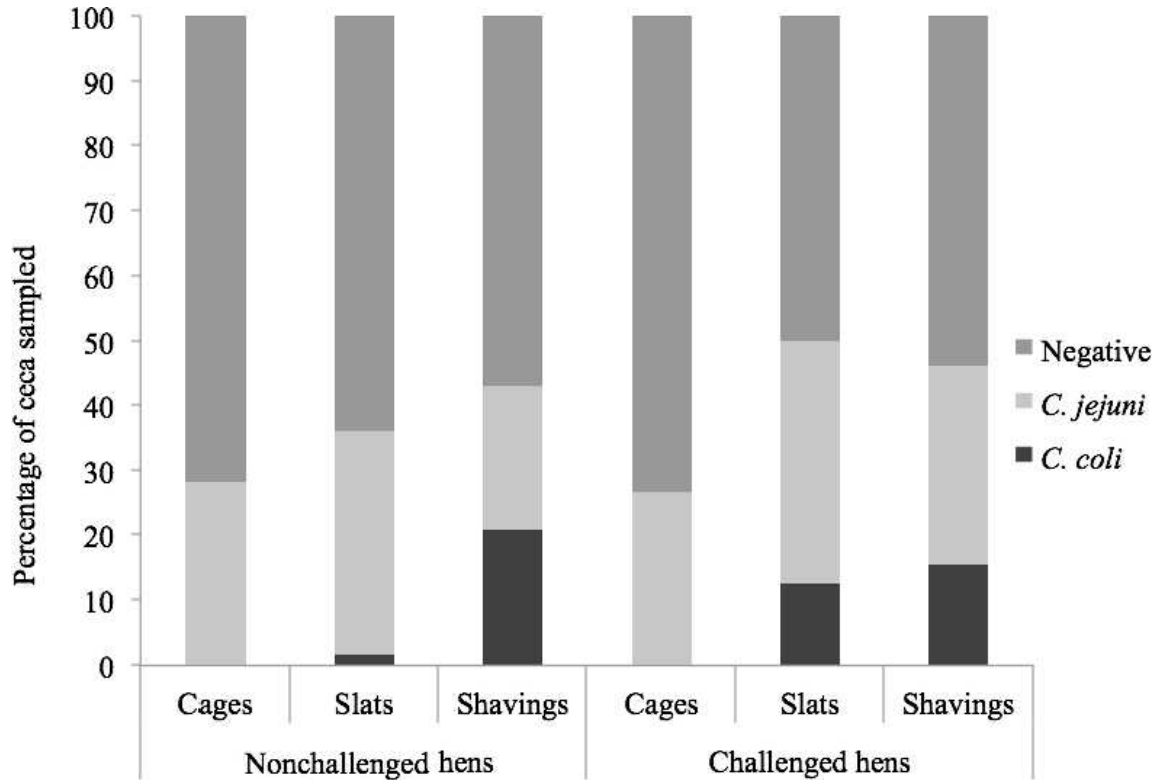

Fig. 1. Percentage of cecum samples collected from nonchallenged and challenged hens in cages, slats, and shavings housing systems positive for *C. coli* or *C. jejuni* or that were negative.

was recovered from slats and shavings housing systems at lower levels than *C. jejuni*. Approximately 2% of the cecum samples collected from nonchallenged hens housed on slats were positive for *C. coli,* and 34% were positive for *C. jejuni*. Among cecum samples collected from nonchallenged hens housed on shavings, 21% and 22% were positive for *C. coli* and *C. jejuni*, respectively. For challenged hens housed on slats, 13% of the cecum samples collected were positive for *C. coli,* and 38% were positive for *C. jejuni*. Among cecum samples collected from challenged hens housed on shavings, 16% and 31% were positive for *C. coli* and *C. jejuni*, respectively. All *Campylobacter* isolated from the spleen, LGB, URT, and ovarian follicle samples were confirmed to be *C. jejuni*. The LRT samples from hens housed in cages and on slats were positive for *C. jejuni*, and one LRT from a hen housed on shavings was positive for *C. coli*.

During trial 1, the cage and shavings pens were positive for *Salmonella* Typhimurium, and the slat pen was positive for *C. coli* via stepped-on drag-swab sampling. During trial 2, the cage, slat, and shavings pens were all positive for *Salmonella* Typhimurium, and only the shavings pen was positive for *C. coli*. All pens in trial 3 were negative for *Salmonella* Enteritidis and *C. coli*, but positive for *Salmonella* Typhimurium (residual from trial 1). *Salmonella* Enteritidis was not recovered from any of the environmental samples (litter and nipple drinker swab samples) taken during trials 4 and 5. During trial 4, *C. jejuni* was recovered from the cage, slat, and shavings pens and the nipple drinkers of the cage pen. The litter of the shavings pen used in trial 4 was also positive for *Salmonella* Typhimurium (residual from trial 2). During trial 5, all pens were positive for *C. jejuni*, and the litter and nipple drinkers in the shavings pen, as well as the nipple drinkers in the slats pen, were positive for *Salmonella* Typhimurium. Results indicate that the sampling methods used were sufficient for recovering *Salmonella* Typhimurium from this environment but might not have been adequate for *Salmonella* Enteritidis.

During trial 4, all eggshell and membrane samples ($n = 21$) from eggs produced by commingled challenged hens (pooled by housing system) were negative for *Salmonella* Enteritidis. During trial 4, *Salmonella* Typhimurium was recovered from one of six eggshell samples from eggs produced on slats and from one of eight eggshell samples from eggs produced on shavings. For trial 5, the eggshell and membrane samples ($n = 11$) from eggs produced by commingled hens were all negative for *Salmonella* Enteritidis and *Salmonella* Typhimurium.

DISCUSSION

The horizontal transmission of *Salmonella* among nonchallenged hens housed in cages, on slats, and on shavings was similar, and *Salmonella* prevalence among all hens was relatively low, ranging from 25% to 38%. This is partially because of the seemingly poor colonization of the *Salmonella* Enteritidis strain used to challenge hens in the last three trials. *Salmonella* Enteritidis was recovered from only one LRT of the 151 tissue organ samples collected from challenged hens inoculated with *Salmonella* Enteritidis. If *Salmonella* Enteritidis did colonize and persist within the cecum of challenged hens, it is likely that the levels of bacteria shed into the environment after commingling were minimal because *Salmonella* Enteritidis was not recovered from any cecum samples collected from nonchallenged hens or environmental samples collected from each housing system. *Salmonella* Typhimurium was recovered only from cecum and LRT samples of challenged hens housed in cages, suggesting that the bacteria did not spread to and colonize within other abdominal organs. Colonization of the cecum could have resulted from either oral or intracolonic inoculation, whereas colonization of the LRT most likely resulted from intravaginal inoculation. However, the oviduct can become contaminated through ascending infection

from the cloaca (20,36). In addition to cecum and LRT samples, *Salmonella* Typhimurium was recovered from the URT, spleen, and LGB samples of challenged hens housed on slats and shavings, indicating that the bacterial infection became systemic in these hens. *Salmonella* Typhimurium did colonize in the LRT and translocate to the URT, suggesting that the contents of eggs produced by challenged hens could be contaminated before oviposition. However, *Salmonella* was not recovered from any of the ovarian follicles sampled, implying that the egg yolks would not likely be contaminated. *Salmonella* Typhimurium colonized within the intestinal tract and translocated to other organs of hens at higher rates than *Salmonella* Enteritidis. Hen age might have contributed to the poorer colonization of *Salmonella* Enteritidis. Laying hens challenged with *Salmonella* Enteritidis were 65, 70, and 72 wk of age (compared with 56 and 61 wk of age for hens challenged with *Salmonella* Typhimurium), and in general, hens with more established intestinal microflora are less susceptible to *Salmonella* colonization. However, *Salmonella* Enteritidis has been recovered from laying hens of a similar age (29). The strain of *Salmonella* Enteritidis used in this study might have been lacking factors needed to proliferate within the intestinal tract of laying hens. The colonization rate of this marker *Salmonella* Enteritidis could be underrepresented because a field strain of *Salmonella* Enteritidis is likely to colonize hens at a higher rate and, therefore, have a greater potential for horizontal transmission. Biosecurity constraints prevented us from using a field strain of *Salmonella* Enteritidis in these animal facilities.

With the inclusion of residual pen *Salmonella* Typhimurium (from trials 1 and 2), the horizontal transmission of *Salmonella* among nonchallenged pen-mate hens was significantly greater in the shavings system than in the caged and slats housing systems. The levels of *Salmonella* Typhimurium excreted through the feces of hens in trials 1 and 2 were sufficient for the bacteria to persist in the environment of each housing system and infect nonchallenged and challenged hens used in subsequent trials 3, 4, and 5. *Salmonella* Typhimurium was recovered from environmental samples through the duration of the study (16 wk). Although residual *Salmonella* Typhimurium was recovered from the cecum of nonchallenged and challenged hens and LRT of challenged hens in each housing system, the largest increase in *Salmonella* prevalence was among hens housed on shavings. Colonization by *Salmonella* Typhimurium occurred although the nonchallenged and *Salmonella* Enteritidis challenged hens were introduced into the pens at the same time, avoiding the potential for precolonization of the nonchallenged hens with environmental *Salmonella* Typhimurium before exposure to the *Salmonella* Enteritidis–challenged hens. It is thought that the risk for disease and pathogen transmission increases among hens housed in cage-free floor systems because they are not separated from their feces (16), and the litter in the all-shavings pen played an important role in the persistence and transmission of *Salmonella* Typhimurium in the current study. Among challenged hens in each housing system, the percentage of cecum and LRT samples positive for both *Salmonella* Typhimurium and Enteritidis were very similar, and therefore ascending infection from the cloaca likely resulted in contamination of the LRT.

Reports regarding the influence of housing system on *Salmonella* prevalence among laying hens have been conflicting. Although some studies have indicated that housing system has no effect on *Salmonella* prevalence in laying hens (24,37), other studies have reported a higher prevalence of *Salmonella* in caged flocks than in cage-free flocks (29,43,44,47). In the present study, *Salmonella* spread minimally among nonchallenged hens housed in cages, and these results suggest that housing laying hens in cages is not a significant specific risk factor for the transmission of *Salmonella*. However, the number of hens used in this study is considerably lower than the number of hens in a commercial facility (5000–350,000 hens/house). Flock size has been reported to have an effect on *Salmonella* prevalence among caged layers (29,34,43) because the number of hens housed in caged facilities is generally larger than the number of hens kept in cage-free facilities.

Salmonella Enteritidis is the primary serovar associated with laying hens and table eggs, and studies have shown that even when laying hens are orally challenged with large doses (10^9 cfu/ml) of *Salmonella* Enteritidis, the incidence of egg contamination is reasonably low (yolk, 2.5%–7%; albumen, 0%–2%) (22,23). In the present study, with eggs produced by challenged hens held in isolation, *Salmonella* Enteritidis was detected in only one pooled eggshell sample, suggesting that at the time of egg collection, *Salmonella* was present on the eggshell(s) or within the eggshell membranes when the egg was collected. The eggshell samples collected from eggs produced in the slats and shavings pens that were positive for *Salmonella* Typhimurium (trial 4) were likely contaminated in the housing system including the nest box. Although residual *Salmonella* Typhimurium was not recovered from the environment of the all-slats pen in trial 4, it was likely present in the environment as the percentage of positive ceca collected from nonchallenged hens increased from 15 to 20 (three additional hens).

The horizontal transmission of *Campylobacter* among nonchallenged pen-mate hens was significantly greater on shavings (47%) than in cages (28%), and for hens housed on slats (36%), transmission was similar to that of both hens housed in cages and on shavings. The litter in the shavings pen contributed to the survival of the *Campylobacter* that was shed through the feces of challenged hens. Coprophagia, or the consumption of feces, contributes to the persistence of *C. jejuni* infections in poultry, and the survival of *Campylobacter* in damp litter prolongs the shedding period of broilers (41). Overall, more ceca were positive for *Campylobacter* than *Salmonella*, indicating that *Campylobacter* might spread to pen- or cage-mates and persist within a flock longer than *Salmonella*. This trend held for *C. jejuni* in all housing systems and *C. coli* in the shavings housing system only. In the current study, *C. jejuni* (81/99; 82%) was recovered from more tissue organ samples than *C. coli* (18/99; 18%), but among naturally infected commercial laying hens, Cox *et al.* (11) found that *C. jejuni* and *C. coli* accounted for 50% and 49%, respectively, of *Campylobacter* isolated and that co-colonization did occurred in <10% of the hens. In each housing system, *Campylobacter* was recovered from both the upper and lower segments of the reproductive tract and the ovarian follicles, whereas *Salmonella* was not. Cox *et al.* (9) also recovered *Campylobacter* (19%) from ovarian follicles of broiler breeder hens at a higher rate than *Salmonella* (1%). Two primary implications are associated with the recovery of *Campylobacter* from reproductive tissues of hens: one is the possible production of contaminated eggs, and the other is the potential for vertical transmission of *Campylobacter* from breeder hens to their progeny (8).

The horizontal transmission of *Salmonella* from challenged hens between caged and cage-free housing systems was not significantly different. However, when residual *Salmonella* Typhimurium was taken into account, the shavings housing system provided the greatest horizontal transmission. The shavings housing system also provided the greatest horizontal transmission of *Campylobacter*. Therefore, with regard to food safety, the overall results of this study indicates that the caged housing system provides the lowest potential for horizontal transmission of *Salmonella* and *Campylobacter* among egg-laying hens.

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