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Colonization of a Marker and Field Strain of *Salmonella* Enteritidis and a Marker Strain of *Salmonella* Typhimurium in Vancomycin-Pretreated and Nonpretreated Laying Hens

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SUMMARY. This study was conducted to evaluate the influence of a vancomycin pretreatment on the ability of marker (nalidixic-acid resistant) *Salmonella* Enteritidis (SE^M), field *Salmonella* Enteritidis (SE^F), and marker *Salmonella* Typhimurium (ST^M) strains to colonize within the intestinal and reproductive tracts and translocate to other organs of leghorn laying hens. In each of three trials, caged laying hens (76, 26, and 33 wk of age) were divided into six groups designated to receive SE^M, SE^F, or ST^M, and half were pretreated with vancomycin ($n = 11–12$ hens). Vancomycin-treated hens received 10 mg vancomycin in saline/kilogram body weight orally for 5 days to inhibit Gram-positive bacteria within the intestines. On Day 6, all hens were concurrently challenged by oral, intravaginal, and intracolonal routes with *Salmonella* and placed into separate floor chambers by *Salmonella* strain. Two weeks postinoculation, all hens were euthanatized and the ceca, spleen, liver/gall bladder (LGB), upper (URT), and lower (LRT) reproductive tracts, and ovarian follicles were aseptically collected, and analyzed for *Salmonella*. Results did not differ for the three hen's ages and were therefore combined. The vancomycin pretreatment also had no significant effect on the colonization ability of SE^M, SE^F, or ST^M, and therefore results were combined within *Salmonella* strain. The marker strain of *Salmonella* Enteritidis was recovered from 21% of ceca, 4% of LGB, 9% of LRT, and 17% of the fecal samples. The field strain of *Salmonella* Enteritidis was recovered from 88% of ceca, 96% of spleen, 92% of LGB, 30% of LRT, 4% of URT, 13% of follicle, and 42% of the fecal samples. The marker strain of *Salmonella* Typhimurium was recovered from 100% of ceca, 74% of spleen, 91% of LGB, 30% of LRT, 9% of URT, 9% of follicle, and 100% of the fecal samples. Among ceca, spleen, LGB, and fecal samples, SE^F and ST^M colonization was significantly greater than SE^M colonization. Overall prevalence of *Salmonella* in the reproductive tracts of challenged hens was relatively low, ranging from 4% to 30%.

RESUMEN. Colonización de una cepa marcadora y de campo de *Salmonella* Enteritidis y de una cepa marcadora de *Salmonella* Typhimurium en gallinas ponedoras pretratadas y no pretratadas con vancomicina.

Este estudio se realizó para evaluar la influencia de un tratamiento previo de vancomicina en la capacidad de una cepa marcadora (SE^M) (resistente al ácido nalidíxico) y de campo (SE^F) de *Salmonella* Enteritidis, y una cepa marcadora de *Salmonella* Typhimurium (ST^M) para llevar a cabo la colonización en los tractos intestinal y reproductivos y para diseminarse a otros órganos en gallinas Leghorn. En cada uno de los tres ensayos, gallinas ponedoras enjauladas (de 76, 26 y 33 semanas de edad) se asignaron en seis grupos designados para recibir SE^M, SE^F, o ST^M, y la mitad fueron tratadas con vancomicina ($n = 11$ a 12 gallinas). Las aves tratadas con vancomicina recibieron 10 mg de vancomicina en solución salina/kg de peso corporal por vía oral durante 5 días para inhibir bacterias Gram-positivas dentro de los intestinos. En el día seis, todas las gallinas fueron desafiadas al mismo tiempo por vía oral, intravaginal, e intracolonal con *Salmonella* y se colocaron en cámaras en piso separadas por cada cepa de *Salmonella*. Dos semanas después de la inoculación, se practicó la eutanasia de todas las gallinas y se recolectaron de manera aséptica los ciegos, el bazo, el hígado/vesícula biliar, los tractos reproductivos superiores e inferiores, y los folículos ováricos, y se analizaron para detectar *Salmonella*. Los resultados no difieren entre las edades de las gallinas y por lo tanto se combinaron. El tratamiento previo con vancomicina tampoco tuvo un efecto significativo sobre la capacidad de colonización de las cepas SE^M, SE^F, o ST^M, y por lo tanto, los resultados se combinaron dentro de las cepas de Salmonella. La cepa marcadora de *Salmonella* Enteritidis marcador se recuperó del 21% de los ciegos, del 4% del hígado/vesícula biliar, del 9% del tracto reproductor inferior y del 17% de las muestras fecales. La cepa de campo de *Salmonella* Enteritidis se recuperó del 88% de los ciegos, del 96% de los bazos, del 92% del hígado/vesícula biliar, del 30% del tracto reproductor inferior, del 4% del tracto reproductor superior, del 13% de los folículos, y del 42% de las muestras fecales. La cepa marcadora de *Salmonella* Typhimurium se recuperó de un 100% de las muestras cecales, del 74% de los bazos, del 91% del hígado/vesícula biliar, del 30% del tracto reproductor inferior, del 9% del tracto reproductor superior, del 9% de los folículos, y del 100% de las muestras fecales. Con relación a las muestras de ciegos, el bazo, hígado/ vesícula biliar y de las muestras de heces, la colonización por SE^F y por ST^M fue significativamente mayor que la colonización por SE^M. La prevalencia general de *Salmonella* en el tracto reproductor de las gallinas desafiadas fue relativamente baja, con un rango entre el 4% a 30%.

Key words: *Salmonella* colonization, vancomycin, cage-free laying hens

Abbreviations: BGS = brilliant green sulfa; BPW = buffered peptone water; cfu = colony-forming units; LGB = liver/gallbladder; LRT = lower reproductive tract; SE^F = *Salmonella* Enteritidis field strain; SE^M = *Salmonella* Enteritidis marker strain; ST^M = *Salmonella* Typhimurium marker strain; URT = upper reproductive tract

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Animals are reservoirs for many zoonotic pathogens, including *Salmonella enterica* (2,3), which is partially because the microorganism can either persist in the animal's intestinal tract or translocate to and invade other abdominal organs (12,20). Invasive pathogens pose a greater threat to food safety, as contamination can be spread beyond the surface and into the interior of a food. An important example of this is egg-associated salmonellosis (20). *Salmonella* infection among laying hens is a food-safety concern for the commercial table egg industry and *Salmonella enterica* serovar Enteritidis is currently the primary cause of egg-associated salmonellosis in humans (6,9,15). Greig and Ravel (15) recently analyzed the international food-borne outbreak data reported between 1988 and 2007 and found that egg-associated outbreaks (584) were due to *Salmonella* Enteritidis (73.7%), other *S. enterica* (15.3%), and *S. enterica* serovar Typhimurium (8.4%). The detection prevalence of *Salmonella* Enteritidis among the contents of eggs produced by naturally infected hens has been reported to be relatively low at $<1.0\%$ (21,27).

Antimicrobial-resistant or marker strains of *Salmonella* have been used in many scientific studies (1,7,17,18), as they generally remain viable and are readily identifiable with simplified cultivation methods. However, the acquisition of mutations in antibiotic target genes, caused by inducing antibiotic resistance, have been associated with fitness costs such as a slower growth rate, reduced virulence, and colonization ability (26). The poor colonization of a *Salmonella* Enteritidis marker strain in a previous study may also have been influenced by hen age (56–72 wk) compared to young broilers (5–7 wk) (16). In general, chickens become more resistant to *Salmonella* colonization with age (from chicks to maturity) and the establishment of intestinal microflora (11,24), but it is unclear how further increases in age beyond maturity may affect *Salmonella* colonization.

The objectives of this study were 1) to compare the colonization ability of *Salmonella* Enteritidis and *Salmonella* Typhimurium marker strains to that of a *Salmonella* Enteritidis field strain, and 2) to evaluate the use of vancomycin antibiotic pretreatment to aid the ability of these *Salmonella* strains to colonize and translocate within a laying-hen *Salmonella* model. Vancomycin pretreatment has been used to enhance *Salmonella* colonization in 4-wk-old broilers (30). Findings from this study would help determine if lowering intestinal microflora by vancomycin pretreatment improves *Salmonella* colonization in mature leghorn laying hens.

MATERIALS AND METHODS

Experimental design and inoculation. In each of three separate trials, leghorn laying hens (Hy-Line W-36) were randomly allocated to one of six treatment groups: 1) nalidixic-acid–resistant *Salmonella* Enteritidis marker (SE^M; provided by N. A. Cox, U.S. Department of Agriculture/Agricultural Research Service, Russell Research Center, Athens, GA) strain with no vancomycin pretreatment, 2) nalidixic-acid–resistant SE^M strain with vancomycin pretreatment, 3) *Salmonella* Enteritidis field (SE^F; provided by N. A. Cox, of different origin than the SE^M) strain with no vancomycin pretreatment, 4) SE^F strain with vancomycin pretreatment, 5) nalidixic-acid–resistant *S.* Typhimurium marker (ST^M; provided by N. A. Cox and previously used by Buhr *et al.* (4) and Cox *et al.* (5)) strain with no vancomycin pretreatment, and 6) nalidixic-acid–resistant ST^M strain with vancomycin pretreatment. Hens were housed in separate isolated chambers in individual wire cages according to their designated vancomycin or nonvancomycin pretreatment. Hens that were allocated to the vancomycin pretreatment groups received 0.5 ml of a vancomycin (Sigma Aldrich, St. Louis, MO) solution (prepared at 10 mg/kg body weight) orally for 5 days to reduce Gram-positive intestinal microflora (19). On the sixth day, all hens were challenged first orally (1 ml), then intravaginally (1 ml), and finally intracolonally (1 ml) (all three routes for each hen to optimize colonization) with either SE^M average 1.1×10^8 colony-forming units (cfu)/ml, SE^F average 2.0×10^9 cfu/ml, or ST^M average 2.4×10^8 cfu/ml. Oral challenge was achieved by placing the tip of a 1-ml syringe in the back of the open mouth, avoiding the glottis. Intravaginal challenge was conducted by eversion of the vaginal simulating artificial insemination with a 1-ml syringe. Intracolonal challenge was attained by placing a 5-cm section of soft plastic tubing onto the 1-ml syringe and then inserting through the cloaca into the colon. After inoculation, hens were placed in separate isolated chambers (7 ft $\times$ 7 ft [2.1 m $\times$ 2.1 m]) on fresh pine shavings with access to a nest box (six nests/box), water, and feed *ad libitum*, and a 16-h photoperiod. A total of three chambers (one each for SE^M, SE^F, and ST^M) were used with nonpretreated and vancomycin-pretreated hens inoculated with the same strain of *Salmonella* placed commingled in the same chamber. The hens used in Trials 1, 2, and 3 were 76, 26, and 33 wk old, respectively. In the first trial (18 hens), 3 hens were used in each treatment group (6 hens/chamber) (8.2 ft^2/hen [0.8 m^2/hen]). In the second trial (30 hens), 5 hens were used in each treatment group (10 hens/chamber) (4.9 ft^2/hen [0.4 m^2/hen]), and in the third trial (24 hens), 4 hens were used in each treatment group (8 hens/chamber) (6.1 ft^2/hen [0.6 m^2/hen]).

Fecal samples. To collect fecal samples, hens from each chamber were placed into individual units of a three-unit portable wire cage system lined with clean brown kraft paper. Approximately 3 g of fresh fecal material was aseptically collected from each hen. After sample collection, hens were returned to their designated chamber. To maintain aseptic technique, new gloves were used for each fecal sample and clean kraft paper was used for each group of hens placed in the portable cage system. Fecal samples were collected 1 wk postinoculation during each trial. Each fecal sample was placed in a sterile 50-ml centrifuge tube and transferred to the laboratory for analysis. A standard volume of 30 ml of sterile buffered peptone water (BPW; 1%; Acumedia, Lansing, MI) was added to each fecal sample, and all samples were vortexed. Samples in BPW for *Salmonella* analysis were incubated at 37 C for 24 hr.

Environmental samples. During each trial, the litter floors of each chamber were sampled by stepped-on drag swabs (n = 1/chamber). Presoaked drag swabs (DS-001, Solar Biologicals, Inc., Ogdensburg, NY) were unwound and dragged across the litter in a figure-eight shape around the chamber (4). Swabs were stepped on five times during sampling with a clean, disposable boot cover that was put on upon entering each chamber. The nipples on each drinker line were also sampled for *Salmonella.* Each nipple (eight/line) was swabbed with an open gauze swab (n = 1/chamber) held in a gloved hand. Individual litter and nipple drinker swab samples were placed in sterile sample bags and transported to the laboratory. One hundred milliliters of BPW was added to each sample. All samples were massaged by hand to loosen any attached debris. Swab samples were incubated at 37 C for 24 hr for *Salmonella* analysis.

Egg samples. Eggs were collected from each chamber daily, placed on a clean cardboard flat, and held in an on-site cooler at 5 C for less than 24 h until sampling. Eggs were pooled by chamber (SE^M, SE^F, and ST^M; Trials 1 and 3, n = 5 eggs/sample; Trial 2, n = 8 eggs/sample), and eggs from vancomycin-pretreated and nonpretreated hens were not distinguished. A total of 129 eggs (23 samples) were collected for hens challenged with SE^M, 152 eggs (25 samples) for hens challenged with ST^M, and 167 eggs (28 samples) for hens challenged with SE^F. Eggs within each pooled sample remained unsanitized and were cracked on individual sections of clean aluminum foil. The internal contents were discarded because of the low prevalence and recovery of *Salmonella* contamination among contents (11,13). The eggshell and adhering membrane complex were crushed by hand and placed in a sterile sample bag. Shell samples from eggs within each pooled sample were combined and placed in the same sample bag. To maintain aseptic technique, new gloves were used between each pooled sample. Sterile BPW was added to each sample bag at a ratio of 20 ml/eggshell and all samples were incubated at 37 C for 24 hr for *Salmonella* analysis.

Organ samples. Two weeks postinoculation, all hens were euthanatized by electrocution for sample collection. The ceca, spleen, liver/gall bladder (LGB), upper (URT: infundibulum, magnum, and isthmus) and lower (LRT: shell gland and vagina) reproductive tracts, and ovarian follicles were aseptically collected from all hens for

Table 1. Percentage of samples positive for *Salmonella* from vancomycin (VNC) pretreated and nonpretreated laying hens.[A]

	n[B]	Cecum	Spleen	LGB	LRT	URT	Follicles	Feces	Litter[C]	Drinkers[C]	Eggshells[D] (%; number)
SE^M no VNC	12	8	0	8	8	0	0	25			
SE^M with VNC	11	36	0	0	9	0	0	9			
Total for SE^M	23	21[aE]	0[a]	4[a]	9	0	0	17[a]	0 (0/3)	0 (0/3)	0[a] (0/23)
SE^F no VNC	12	83	100	100	17	0	17	42			
SE^F with VNC	12	92	92	83	42	8	8	42			
Total for SE^F	24	88[b]	96[b]	92[b]	30	4	13	42[b]	76 (2/3)	76 (2/3)	75[b] (21/28)
ST^M no VNC	11	100	73	91	18	18	18	100			
ST^M with VNC	12	100	75	83	42	0	0	100			
Total for ST^M	23	100[b]	74[b]	91[b]	30	9	9	100[c]	100 (2/3)	100 (3/3)	72[b] (18/25)

[A]SE^M = *Salmonella* Enteritidis marker strain, SE^F = *Salmonella* Enteritidis field strain, ST^M = *Salmonella* Typhimurium marker strain, LGB = liver/gallbladder, LRT = lower reproductive tract (shell gland and vagina), URT = upper reproductive tract (infundibulum, magnum, and isthmus).

[B]$n = 11$ hens because 1 hen from the respective pretreatment group died during Trial 2.

[C]Litter sampled via stepped-on drag swab and nipples of drinker line sampled with open gauze swab were sampled once per trial ($n = 3$). Parentheses indicate number positive of total number of samples.

[D]Egg samples were pools (Trials 1 and 3, $n = 5$ eggs/sample; Trial 2, $n = 8$ eggs/sample).

[E]Percentages within columns with different lowercase-letter superscripts are significantly different ($P < 0.05$).

Salmonella 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 smashed with a rubber mallet to expose the internal contents of the samples. BPW was added at a ratio of three times the weight of the sample (ml/g). All samples were then placed in a Stomacher 400 (Fisher Scientific, Hampton, NH) and stomached for 1 min. Samples were then incubated at 37 C for 24 hr.

Plating procedures. *Salmonella* were isolated as described previously (1,4). Following incubation, two loops (20 µl) from each sample for SE^M and ST^M analyses were streaked onto brilliant green sulfa (BGS) agar (Acumedia, Lansing, MI) containing 200 ppm nalidixic acid (Sigma Aldrich, St. Louis, MO). BGS plates were incubated for 24 h at 37 C. Colonies characteristic of *Salmonella* were selected and subjected to the slide agglutination tests with the use of *Salmonella* O Antisera (Becton Dickinson, Sparks, MD) for serogroup (A-I followed by Group D_1 for SE^M and Group B for ST^M) confirmation. For SE^F samples, 0.1 ml of incubated BPW was transferred to 9.9 ml of Rappaport-Vassiliadis (RV; Becton Dickinson, Sparks, MD) broth and 0.5 ml of incubated BPW was transferred to 9.5 ml of tetrathionate (TT; Becton Dickinson, Sparks, MD) broth. The RV and TT broths were incubated at 42 C for 24 hr. Two loops (20 µl) from the incubated RV and TT broths were streaked onto BGS and xylose–lysine–tergitol 4 (XLT4; Acumedia, Lansing, MI) plates, and all plates were incubated at 37 C for 24 hr. Suspect colonies were picked and transferred to triple sugar iron (TSI; Becton Dickinson, Sparks, MD) and lysine iron agar (LIA; Acumedia, Lansing, MI) slants. Slants were incubated at 37 C for 24 hr. Presumptive colonies were then subjected to slide agglutination tests with the use of *Salmonella* O Antisera for serogroup (A-I followed by Group D_1 for SE^F) confirmation.

Statistical analysis. Chi-square and Fisher's exact test were used to identify differences in *Salmonella* colonization due to bacterial strain (3; SE^M, SE^F, and ST^M) and vancomycin pretreatment (2; nonpretreated and pretreated hens), and hen's age (3; 26, 33, and 76 wk). Because analysis determined that neither vancomycin pretreatment nor hen's age had any significant impact on colonization for any of the three *Salmonella* strains, the nonpretreatment and pretreatment data and hen's age data were combined and reanalyzed by *Salmonella* strain only. Differences were considered significant at $P < 0.05$.

RESULTS

There was no significant difference ($P > 0.05$) in SE^M, SE^F, and ST^M colonization (Table 1) between nonpretreated and vancomycin pretreated hens or hen age 26, 33, or 76 wk for all samples collected. At 1 wk postinoculation SE^M was recovered from 17% (4/23) of the hen's fecal samples, whereas SE^F was recovered at significantly higher rate from 42% (10/24) of the hen's fecal samples, and ST^M was recovered from 100% (23/23) of the hen's fecal samples. For SE^F- and ST^M-challenged hens colonization was significantly greater among cecum, spleen, and LGB samples than SE^M-challenged hens. SE^M was recovered from 21% of cecum, 4% of the LGB, and 9% of the LRT samples, and all positive samples were collected from different hens. SE^F was recovered from 88% of the cecum, 96% of the spleen, 92% of the LGB, 30% of the LRT, 4% of the URT, and 13% of the follicle samples. Six out of the seven LRT samples that were positive for SE^F were collected from hens that also had positive ceca. The URT (1) and ovarian follicle (3) samples that were positive for SE^F were collected from four different hens. *Salmonella* Typhimurium was recovered from 100% of the cecum, 74% of the spleen, 91% of the LGB, 30% of the LRT, 9% of the URT, and 9% of the follicle samples. The two URT samples that were positive for ST^M were collected from hens that had positive LRT samples. Of the two ovarian follicle samples that were positive for ST^M, one was collected from a hen with positive LRT and URT samples, and the other was collected from a hen with negative LRT and URT samples. SE^M was not recovered from any of the litter or nipple drinker swab samples taken throughout the study (Table 1; $n = 3$). SE^F was recovered from the litter and nipple drinker swab samples taken during Trials 1 and 3, but samples taken during Trial 2 were negative for SE^F. ST^M was recovered from drag swab samples taken during Trials 2 and 3 and nipple drinker swab samples taken during all three trials. All pooled eggshell samples ($n = 23$) from eggs produced by hens challenged with SE^M were also negative (Table 1), whereas eggs produced by hens challenged with SE^F had eggshell samples that were 75% positive (21/28), and the eggs produced by hens challenged with ST^M had eggshell samples that were 72% positive (18/25). These results indicate that the sampling methods used were sufficient for recovering ST^M and SE^F from the chamber environment. However, SE^M was unable to colonize and disseminate sufficiently following laying hen challenge.

DISCUSSION

Vancomycin is effective against Gram-positive bacteria, and studies have shown that Gram-positive bacteria are abundant in and predominately cultured from the avian intestinal tract (14,23,28).

Reducing established intestinal microflora should make the hen's intestinal tract more susceptible to *Salmonella* infection/colonization. However, in this study, vancomycin pretreatment had no significant effect on the ability of these three *Salmonella* strains to colonize within the ceca and reproductive tracts and to translocate to other organs of the laying hens. These results suggest that both hen age (26, 33, and 76 wk) and the presence of established intestinal microflora were not significant influential factors affecting colonization by these three *Salmonella* strains. Therefore, the SE^M strain used in this study lacked factors needed to colonize and proliferate within the intestinal tract of the laying hens.

SE^F and ST^M colonization was significantly greater among cecum, spleen, and LGB samples than SE^M colonization. However, there was no significant difference in SE^M, SE^F, and ST^M colonization of the reproductive tract due to the overall low recovery $<30\%$. SE^M was recovered from the feces of four hens and the organs of seven separate hens, suggesting that intestinal colonization did occur in approximately 50% (11/23) of the hens. However, it is likely that among the hens fecally excreting SE^M, the bacterial infection did not spread to other organs as the bacteria were not recovered from any organ samples collected. SE^M was primarily recovered from ceca (21%) and LRT (9%) samples. Colonization of the cecum can be attributed to the oral and/or intracolonal routes of inoculation, whereas colonization of the reproductive tract most likely resulted from intravaginal inoculation, although the oviduct can become contaminated through ascending infection from the cloaca (8,25). The SE^M strain was recovered from the LGB of one hen (4%), suggesting that the bacterial infection became systemic for this hen. SE^F was recovered from cecum, spleen, LGB, LRT, URT, and follicle samples, indicating that this strain of *Salmonella* Enteritidis was more invasive than the SE^M strain. SE^F was recovered from 21/24 cecum samples, 22/24 spleen samples, and 23/24 LGB samples. Similar results were reported by Gast and Beard (10), who, after orally challenging laying hens (ranging from 20 to 88 wk), recovered *Salmonella* Enteritidis from 21/25 cecum samples, 20/24 spleen samples, and 19/24 liver samples 2 wk postinoculation. In the current study, all positive URT and ovarian follicle samples were collected from hens with positive spleen and LGB samples. These results suggest that the SE^F translocates to the URT and then disseminates to the ovarian follicles via the vascular system, and that the yolks and contents of eggs produced by the infected hens could become contaminated prior to oviposition. ST^M was also recovered from cecum, spleen, LGB, LRT, URT, and follicle samples. The SE^F and ST^M strains used in this study seemed to be equal in their ability to colonize the ceca and reproductive tracts and to translocate to other organs of laying hens. Keller *et al.* (22) also found that *Salmonella* Enteritidis had no selective advantage over *Salmonella* Typhimurium (field strains) in its capacity to invade reproductive tissues.

The levels of SE^F excreted through the feces of hens in Trials 1 and 3 were sufficient enough for the bacteria to be detected in the environment (litter and drinkers) and ST^M was recovered from environmental samples in all three trials. Environmental contamination likely contributed to the increased rate of SE^F (21/28 egg pools, 75%) and ST^M (18/25 egg pools, 72%) recovery from eggshell samples. Hens used in this study were housed in cages prior to placement into floor chambers and, without being acclimated to or familiar with nesting behavior, they laid the majority of their eggs on the shavings covered floor in a communal nest area. Therefore, eggshells were susceptible to environmental surface contamination and contamination from the hens' feet.

Hen age (26, 33, and 76 wk) and vancomycin pretreatment to reduce established intestinal microflora had no significant effect on *Salmonella* colonization and dissemination. The dosage of vancomycin (10 mg/kg body weight orally for 5 days) given to these laying hens may have been inadequate to demonstrate significantly improved *Salmonella* colonization. Single doses of vancomycin from 10 to 25 mg given to 4-wk-old broiler was adequate for colonization by marker *Salmonella* spp. (Montevideo and Heidelberg) when sampled 1 and 2 wk postchallenge, although no apparent dose response was noted (30). The SE^M strain did not colonize well within the laying hens and was not subsequently recovered from the environmental or eggshell samples. This lack of colonization and dissemination for SE^M may have been due to this strain being repeatedly cultured within the lab without having been passed through a chicken alimentary tract recently. Another possibility may be that when in competition with other bacteria for nutrients during culture, this SE^M is difficult to recover (29). Both SE^F and ST^M colonized the ceca, spleen, and LGB at significantly higher rates than SE^M, and were recovered from environmental and eggshell samples. SE^F and ST^M persisted over the 2-wk postchallenge period within the hens' tissues and the environment (70%–100% of samples), whereas the prevalence of *Salmonella* in the reproductive tracts of these challenged hens was lower, ranging from 4% to 30%. The equivalent colonization and recovery of the ST^M to that of SE^F indicates that the acquisition of resistance to nalidixic acid is not the sole reason that SE^M was a poor colonizer.

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