

## Effect of *Salmonella* Vaccination of Breeder Chickens on Contamination of Broiler Chicken Carcasses in Integrated Poultry Operations<sup>∇†</sup>

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Received 3 June 2010/Accepted 20 September 2010

While measures to control carcass contamination with *Salmonella* at the processing plant have been implemented with some success, on-farm interventions that reduce *Salmonella* prevalence in meat birds entering the processing plant have not translated well on a commercial scale. We determined the impact of *Salmonella* vaccination on commercial poultry operations by monitoring four vaccinated and four nonvaccinated breeder (parental) chicken flocks and comparing *Salmonella* prevalences in these flocks and their broiler, meat bird progeny. For one poultry company, their young breeders were vaccinated by using a live-attenuated *Salmonella enterica* serovar Typhimurium vaccine (Megan VAC-1) followed by a killed *Salmonella* bacterin consisting of *S. enterica* serovar Berta and *S. enterica* serovar Kentucky. The other participating poultry company did not vaccinate their breeders or broilers. The analysis revealed that vaccinated hens had a lower prevalence of *Salmonella* in the ceca (38.3% versus 64.2%;  $P < 0.001$ ) and the reproductive tracts (14.22% versus 51.7%;  $P < 0.001$ ). We also observed a lower *Salmonella* prevalence in broiler chicks (18.1% versus 33.5%;  $P < 0.001$ ), acquired from vaccinated breeders, when placed at the broiler farms contracted with the poultry company. Broiler chicken farms populated with chicks from vaccinated breeders also tended to have fewer environmental samples containing *Salmonella* (14.4% versus 30.1%;  $P < 0.001$ ). There was a lower *Salmonella* prevalence in broilers entering the processing plants (23.4% versus 33.5%;  $P < 0.001$ ) for the poultry company that utilized this *Salmonella* vaccination program for its breeders. Investigation of other company-associated factors did not indicate that the difference between companies could be attributed to measures other than the vaccination program.

Poultry has been estimated to account for nearly 17% of food-borne outbreaks associated with *Salmonella* in the United States (2). The continuing problem of contamination of retail poultry products with *Salmonella* has important public health implications, especially considering the global increase in chicken consumption (2, 27, 33). In response, the U.S. Department of Agriculture (USDA) implemented the Hazard Analysis of Critical Control Points (HACCP) program in meat-processing plants to offer quality control and surveillance in order to reduce the amount of *Salmonella* contamination associated with poultry (1).

The understanding of *Salmonella* transmission within poultry companies is complex due to the size and integrated nature of this food production system. In the United States, most poultry companies oversee the majority of production processes, from (i) the purchase and placement of day-of-age,

breeder bird stocks (broiler breeder) on farms; (ii) contractual arrangements with poultry farmers to raise breeder stock or their progeny meat birds (broiler) themselves; (iii) the production of poultry feed; (iv) the distribution of birds, feed, and veterinary care to contract farmers; (v) the incubation and hatching of broiler meat birds; (vi) the transport of birds to the processing plant; and (vii) the final production of and marketing of poultry meat. *Salmonella* can enter into this commercial system at any point and be transmitted through the integrated farm continuum. While chickens can acquire *Salmonella* from the poultry house environment, feed, rodents, or insects or through direct contact between infected and uninfected birds (horizontal transmission), many *Salmonella* serotypes are egg transmitted, passed from grandparents to breeder stock to meat birds (5, 26, 28, 32). However, on-farm sanitation and rodent control address only the horizontal transmission of *Salmonella* in poultry. In order to effectively reduce *Salmonella* contamination of poultry, the surveillance-and-intervention strategy must include investigation and identification of management factors that affect the presence of these pathogens at all levels of poultry production.

While many different measures have been recommended for the control of *Salmonella* in poultry, vaccination is likely to have a central role in the reduction of *Salmonella* in commercial operations (37), because research studies indicate that it

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† Supplemental material for this article may be found at <http://aem.asm.org/>.

∇ Published ahead of print on 1 October 2010.

may reduce both the horizontal and vertical transmission of *Salmonella* (10, 11, 18–25, 28, 30, 31, 36, 38). Vaccination works by reducing the prevalence of *Salmonella* in breeder hens and their progeny (19, 23, 25) or by increasing the passive immunity of meat birds and blocking the horizontal transmission of *Salmonella* to broiler chickens (22, 25, 35). However, there have been few studies focusing on whether the vaccines are effective in reducing *Salmonella* on a commercial scale (13, 14, 16, 17, 34, 39).

Commercial poultry operations that have adopted *Salmonella* vaccine programs generally use killed vaccines alone or a combination of live and killed *Salmonella* vaccines. The advantage of live-attenuated vaccines is that attenuated *Salmonella* bacteria replicate, colonize, and invade intestinal and visceral organs of inoculated chickens (7), producing long-lasting protective immunity (11). The first live vaccine licensed in the United States for poultry, Megan VAC, is a *Salmonella enterica* serovar Typhimurium  $\Delta$ *cya*  $\Delta$ *crp* mutant, attenuated in order to reduce its ability to infect and persist in the host while still eliciting humoral and cell-mediated immunity against homologous and heterologous serotypes (10, 11, 20, 22, 24). This *Salmonella* strain is avirulent, stable, and immunogenic and does not promote the development of a *Salmonella* carrier state in chickens (22).

While less effective than live vaccines in producing broad serogroup immunity, bacterins have been shown to reduce egg (19, 38) and cecal (28) colonization with *S. enterica* serovar Enteritidis. The objective of this study was to determine the impact of *Salmonella* vaccination of pullet flocks on the transmission of *Salmonella* to their broiler progeny. We compared *Salmonella* prevalences in parental birds (broiler breeder chickens) and their progeny meat birds (broiler chickens) from two collaborating poultry company cohorts and determined the effect of a *Salmonella* vaccination program on *Salmonella* prevalence in meat birds.

#### MATERIALS AND METHODS

**Collaborating companies and study design.** Two integrated commercial poultry companies operating in Northeast Georgia participated in a 2-year-long sampling plan designed to evaluate the prevalence and transmission of *Salmonella* from breeders to broiler carcasses (meat birds). One of the companies adopted a vaccination protocol for pullet flocks, young birds intended for breeding, involving multiple exposures to a live-attenuated vaccine derived from *S. Typhimurium* (serogroup B), followed by injection with a *Salmonella* bacterin consisting of *S. enterica* serovar Berta (serogroup D1) and *S. enterica* serovar Kentucky (serogroup C2). Pullets were exposed to Megan VAC-1 (Lohmann Animal Health, Winslow, ME) by aerosol spray at 1 day of age, at 2 weeks of age, and again through the drinking water at 5 weeks of age. *Salmonella* bacterin (Lohmann Animal Health) was prepared as an oil emulsion vaccine, and 0.5 ml was injected intramuscularly (pectoral muscle) into pullet chickens at 10 and 18 weeks of age (41). The vaccination program was limited to the pullet flocks from this company. The company that adopted this vaccine protocol will be referred to as “company VAX” throughout the manuscript. Company VAX started implementing their *Salmonella* vaccination program in February 2006. The other collaborating company will be referred to as “company NO-VAX.”

Samples were collected from four pullet farms on the day the breeders were placed as chicks on the pullet farms, and once a month afterwards, until the birds were moved to the broiler breeder farms at 18 weeks of age. Once birds have reached sexual maturity (~18 weeks of age), they are referred as broiler breeders and start producing fertile eggs that yield their broiler progeny. One to two flocks of broiler breeders from each original pullet farm were monitored over time: broiler breeder farms receiving these flocks were sampled before birds were placed (environmental samples) and monthly once the birds were present. Four broiler flocks that were hatched from eggs acquired from these broiler breeder

flocks were also included in the study, for a total of four progeny flocks per broiler breeder flock. One broiler flock from company VAX was dropped from the study because birds were mixed with the progeny of broiler breeders not participating in the study, and therefore, 15 (instead of 16) flocks were monitored for that company. Broiler farms were visited during placement at 2 weeks and at 5 weeks of age. Preevisceration samples were also collected at the processing plant during the slaughter of the broiler breeders and broilers. Samples were collected from the two poultry integrators from February 2006 to December 2007.

**Sample collection and detection of *Salmonella*.** Samples were collected and cultured by using previously reported methodologies (26) and as described below.

At the time of pullet placement, the following samples were collected from each house (two to eight houses per pullet farm): chick box liners ( $n = 30$ ), litter drag swabs ( $n = 5$  per house), dust swabs ( $n = 2$  inside each house and  $n = 1$  outside each house), and feed ( $n = 1$ ). With the exception of chick box liners, the same numbers and types of sample were collected on each monthly visit to the pullet houses. From broiler breeder farms, litter drag swabs ( $n = 2$ ), dust swabs ( $n = 3$ ), and feed samples ( $n = 1$ ) were also collected monthly. Additionally, two slat swabs were collected in front of the hen nest boxes at each broiler breeder farm visit. When the broiler breeder flocks were sent out for processing, 30 birds from each flock were collected, and ceca and reproductive tracts were removed for culture. Sampling on broiler farms was similar to that described above for pullet farms, except that after placement, samples were acquired when the birds were 2 and 5 weeks of age. Additionally, 30 preevisceration carcasses from each broiler flock were collected at the processing plant for culture.

Chick box liners were collected and placed into sterile bags stored at 4°C. The surface of each chick box liner was wiped with a drag swab. Swabs consisted of sterile gauze pads soaked with skim-milk solution (9), which were dragged across the birds' bedding material (litter drag swabs), wiped along fan blades (dust swabs), or wiped along the hen boxes or slats (slat swabs). All swabs were placed into 100 ml of tetrathionate brilliant green broth (TTBG) containing 2 ml of iodine (Difco, Division of Becton, Dickinson, and Co., Sparks, MD) (24, 26) and incubated at 41.5°C for 18 h (8). Feed samples were collected from the open hopper below the feed auger. Twenty-five grams of feed was placed into 225 ml of TTBG containing 4 ml of iodine and incubated as described above.

Thirty broiler chicken carcasses were collected at the processing plant for each broiler and breeder flock. The chicken carcasses were pulled from the processing line before the evisceration step, placed into 114-liter-capacity Polar 120 chest coolers (Iglu Co., Katy, TX) packed with ice, and transported back to the Poultry Diagnostic and Research Center (University of Georgia, Athens, GA). The chicken ceca were aseptically removed from each carcass; placed into sterile, 10- by 21-cm, Nasco Whirl Pak bags (Zefon International, Ocala, FL) containing 10 ml sterile phosphate-buffered saline (PBS); and macerated for 5 min using a stomacher (Tekmar Co., Cincinnati, OH). One hundred milliliters of TTBG containing 2 ml of iodine was added to the cecum homogenate, and the mixture was incubated at 41.5°C for 18 h.

Because broiler breeder flocks are reproductively efficient for only 12 to 14 months before egg production declines, many companies replace broiler breeder flocks yearly and process the older flocks for human consumption. For each broiler breeder flock that was processed (~65 weeks of age), we collected carcasses from the processing plant prior to the evisceration step. The broiler breeder carcasses were transported and processed as described above for the broiler chicken steps, with a few modifications described below. Both the ceca and reproductive tracts were isolated aseptically from the hen carcasses and placed into separate Whirl-Pak bags. The hen carcasses and internal organs were significantly larger than those of the broilers; therefore, 50 ml of sterile PBS was added to each Whirl-Pak bag prior to the maceration step with the stomacher. Fifty milliliters of double-strength TTBG containing 2 ml iodine was added to each organ homogenate, and samples were incubated at 42°C for 18 h.

***Salmonella* isolation.** Following overnight incubation of TTBG broth at 41.5°C, a loopful of the enrichment culture was streaked for colony isolation on XLT4 plates, and the plates were incubated at 37°C overnight. A single, black, H<sub>2</sub>S-positive colony was streaked onto tryptic soy agar, which was incubated at 37°C overnight. The XLT4 plates were incubated for another 24 h at room temperature in order to detect any additional, slow-H<sub>2</sub>S-producing colonies. Presumptive positive colonies were confirmed as *Salmonella* colonies by slide test agglutination using polyvalent *Salmonella* serogroup A-I Vi antisera (Difco). *Salmonella* isolates were placed into freezer stock medium (1% peptone, 5% glycerol) and stored at -80°C.

**Survey of farming practices during farm visits.** Investigators made 239 farm visits to the 49 farms (~24 farms/company) participating in the study between February 2006 and December 2007. During each visit, the investigators com-

TABLE 1. Correlation between vaccination of pullet flocks and *Salmonella* prevalence in broiler chicken meat birds for two poultry integrators

Bird and sample	Company NO-VAX			Company VAX			P value <sup>a</sup>
	No. of samples	No. of positive samples	% positive samples	No. of samples	No. of positive samples	% positive samples	
<b>Pullets</b>							
Chick box liners	782	0	0.0	359	3	0.8	<b>0.011</b>
Litter swabs	670	270	40.3	347	141	40.6	0.918
Feed	117	3	2.6	64	2	3.1	0.833
Dust <sup>b</sup>	421	58	14.4	226	18	8.0	<b>0.029</b>
Total	1,990	331	16.8	996	164	16.5	0.908
<b>Broiler breeders</b>							
Litter/slat swabs	256	83	32.4	242	85	35.1	0.738
Feed	7	1	14.3	42	5	11.9	1.000
Dust <sup>b</sup>	178	10	5.6	184	10	5.4	0.939
Carcasses	299	172	57.5	364	93	25.5	<b>&lt;0.001</b>
Total	740	266	35.9	832	193	23.2	<b>&lt;0.001</b>
<b>Broilers</b>							
Chick box liners	513	172	33.5	474	86	18.1	<b>&lt;0.001</b>
Litter swabs	269	81	30.1	236	34	14.4	<b>&lt;0.001</b>
Feed	54	3	5.6	47	0	0.0	0.246
Dust <sup>b</sup>	161	8	5.0	141	0	0.0	<b>0.008</b>
Carcasses	510	171	33.5	449	105	23.4	<b>0.005</b>
Total	1,507	435	28.9	1,347	225	16.7	<b>&lt;0.001</b>
Company total	4,237	1,032	24.4	3,175	582	18.3	<b>&lt;0.001</b>
Study total				7,412	1,614	21.8	

<sup>a</sup> P value for the chi-square test comparing the numbers of positive samples between the two companies. Boldface type indicates statistical significance.

<sup>b</sup> Samples included dust inside the houses, outside the houses, on ventilation fans, and on feed.

pleted a checklist of farm conditions, farming practices, and house and flock health (see Table S1 in the supplemental material). However, during the study period, an outbreak of infectious laryngotracheitis (ILT) and enhanced biosecurity among poultry farms limited access to some of the farms during the outbreak and reduced interactions with farmers during visits. Therefore, not all visits rendered reliable checklists for analysis. Table S1 in the supplemental material provides the variables from the 179 completed checklists entered into a Microsoft Excel spreadsheet (Microsoft Office 2007).

**Data analyses.** All data were analyzed by using Intercooled Stata 9.2 for Windows (Stata Corp. LP). Two data sets were maintained; the first contained the culture results of individual samples.

For each sample, information was kept regarding the flock of origin (company, farm, and house), the date and sequential visit number, the type of sample (litter drag swab and feed, etc.), and the presence or absence of *Salmonella* after culture and confirmation by the agglutination test, which was recorded as a binary outcome (a score of "1" when *Salmonella* was detected by the methods described above and a score of "0" otherwise). The frequencies of positive cultures on farms were compared between companies by using a chi-square test of homogeneity (12).

The second database contained the results of the farm checklists for each visit by investigators. For each farm visit, checklist information was recorded for each item listed in Table S1 in the supplemental material. The presence or absence of *Salmonella* at each visit was also recorded as a binary variable, with a score of "1" being assigned to farm visits in which at least one of the samples collected was positive for *Salmonella* and a score of "0" otherwise. The frequencies of binary and categorical variable results representing farming practices were compared between companies by using the chi-square test of homogeneity (12). The association between farming practices and the odds of at least one sample being positive for *Salmonella* on a farm visit was assessed by using a logistic model (12).

The proportion of all samples positive for *Salmonella* at each farm visit was recorded, as was the proportion positive for each type of sample. In addition, the proportion of all samples collected from each farm that were positive for *Sal-*

*monella* during the entire study was recorded. All of these variables were used in different logistic models (12) in which the independent variables included the company of origin and the proportion of positive samples in pullet and broiler breeder farms of origin, and the dependent variable was the odds of *Salmonella* contamination per broiler carcass sample.

## RESULTS

**Prevalence of *Salmonella*-positive samples in vaccinated and nonvaccinated broiler breeder chickens and their lineages.** A total of 7,412 samples were collected, and 1,614 of these (21.8%) were positive for *Salmonella* from the 49 farms and 239 farm visits. The total numbers of samples by company, type of bird, and type of sample as well as the numbers of *Salmonella*-positive samples are shown in Table 1.

The overall proportion of positive samples was lower for the company that vaccinated pullets (18.3% versus 24.4% for the company not using vaccination;  $P < 0.001$ ). We did not observe a statistically significant difference in *Salmonella* prevalence among all samples collected at pullet farms: 16.8% for company NO-VAX versus 16.5% for company VAX ( $P = 0.908$ ). However, we did detect a significant difference in the numbers of *Salmonella*-positive dust samples collected from pullet houses: 14.4% for company NO-VAX versus 8.0% for company VAX ( $P = 0.029$ ). During placement, 3 of 359 chick box liners collected from company VAX were positive for *Salmonella*, but no *Salmonella* was detected in any of the 782 chick

box liners collected from company NO-VAX ( $P = 0.011$ ). For both companies at least one environmental sample was positive for *Salmonella* in two out of the four pullet farms surveyed before the birds were placed. All pullet farms were positive for *Salmonella* by the time the pullet flocks were transferred to broiler breeder farms.

For both poultry integrators, at least one sample (drag swab, slat, or dust) collected from the house environment was positive for *Salmonella* in three out of the four broiler breeder farms surveyed prior to the placement of the new broiler breeder flock. Eventually, all broiler breeder farms in this study were positive for *Salmonella*. There were no statistically significant differences in environmental *Salmonella* detected for the two companies at the broiler breeder level (Table 1). No significant differences in the numbers of *Salmonella*-positive samples from feed and other samples collected were observed. However, the prevalence of *Salmonella* in broiler breeder carcasses was significantly higher for company NO-VAX than for company VAX for the cecum samples (64.2% versus 38.3%;  $P < 0.001$ ) and the reproductive tract samples (51.7% versus 14.2%;  $P < 0.001$ ).

Day-old broiler chickens from company NO-VAX had a higher level of *Salmonella* contamination at the time of placement, with 33.5% of the chick box liners from this company being positive for *Salmonella*, compared with 18.1% for company VAX ( $P < 0.001$ ). Litter drag swabs and dust samples also indicated a significantly higher percentage of environmental contamination with *Salmonella* for company NO-VAX (Table 1). Low-level to no *Salmonella* contamination was observed for the feed of the two poultry companies. Six broiler chicken farms of company NO-VAX (from a total of 16 farms) had at least one environmental sample (litter drag swab or dust) positive for *Salmonella* at broiler bird placement, whereas only one broiler chicken farm (of 15 farms evaluated) was positive at company VAX ( $P < 0.083$ ). Broiler chickens removed from the slaughterhouse and evaluated upon necropsy had a higher percentage of *Salmonella*-positive samples for company NO-VAX (33.5% versus 23.4%;  $P = 0.005$ ).

At the farm level, *Salmonella* was detected in at least one sample for 56 of the 69 farm visits of pullets and broiler breeder flocks of company VAX (81%), whereas *Salmonella* was detected for 62 of the 68 visits of company NO-VAX samples (91%), a difference not significant at the 95% confidence level ( $P = 0.086$ ). These visits included visits to processing plants, at which all flocks of spent hens had at least one *Salmonella*-positive sample. *Salmonella* was detected in at least one sample of broiler flocks from 32 of the 60 farm visits for company VAX (53%) and 56 of the 64 farm visits for company NO-VAX (72%) ( $P = 0.030$ ). No *Salmonella* was detected in chickens collected at the slaughterhouses from one flock of company NO-VAX and two flocks of company VAX.

In evaluating the odds of *Salmonella* contamination of chicken carcasses, the company of origin was the only significant predictor (odds ratio of 0.60 for company VAX compared to company NO-VAX;  $P < 0.001$ ). We could not correlate *Salmonella* prevalence in broiler chicken carcasses with either pullet farm of origin or broiler breeder farm of origin, and no significant correlations could be drawn when the analyses were repeated using either overall *Salmonella* prevalence in broilers

(farm environment and birds) or *Salmonella*-positive chick box liners as the dependent variables.

**Comparison of farming practices.** From the 179 checklists considered completed and included in the data analysis, 100 referred to visits to farms of company VAX and 79 referred to visits to farms of company NO-VAX. A comparison of farming practices between the two companies did not reveal any significant difference regarding the presence of animals outside the houses (animals were observed for 56.4% of the farm visits throughout the study, dogs were observed for 37.8% of visits, and cattle were observed for 25.5% of visits), the presence of open fields close to the houses (55.7% overall), the presence of insects (20.7%), the presence of rodents (5.5%), the water system used (49% used bells, and 51% used nipple systems), the feeding system (pans used at all farms), litter conditions (litter was considered wet by investigators for 19.5% of all visits), temperature inside the house (temperature was considered hot for 23.4% of the visits, cold for 0.6% of the visits, and comfortable otherwise), whether female and male birds were kept in the same house (in 92% of all farms), whether birds were showing signs of stress according to the investigators' judgment (5.3% overall), and whether the investigators could notice any signs of illness (investigators identified signs of illness for 8.8% of all visits performed for both companies).

The company NO-VAX farms were more likely to have weed overgrowth around poultry houses (38.9% versus 15.3% for company VAX;  $P < 0.001$ ), to have old equipment around the houses (45.9% versus 24.7% for company VAX;  $P = 0.002$ ), to have disinfecting footbaths at the poultry house entrance (72.5% versus 2.4% for company VAX;  $P < 0.001$ ), and to use fan and evaporative cooling as opposed to fan-only ventilation (29.2% versus 5% for company VAX;  $P = 0.03$  [this value was compared among farms rather than among farm visits]). Additionally, farm personnel were more likely to use biosecurity garments (boots and coveralls, etc.) for company NO-VAX (23.6% versus 7.0% for company VAX;  $P = 0.025$ ), and investigators judged poultry houses of company NO-VAX as being "tight" (73.5% versus 59.8% for company VAX;  $P = 0.048$ ). To judge whether a house was considered "tight," investigators evaluated the house for the presence of holes in the walls or screens. When assessing the association between these farm practices and the risk of *Salmonella* contamination, controlling for the company of origin (as described in Materials and Methods), only the observation of "nontight" houses had a higher odds (odds ratio = 0.36 compared to "nontight";  $P = 0.013$ ) of detection of at least one *Salmonella*-positive sample during the visit to a specific farm.

## DISCUSSION

HACCP was implemented in an attempt to improve food safety and reduce human illnesses attributable to poultry by mandating in-plant changes that would reduce contamination of the finished, raw product with food-borne pathogens. While chicken carcass contamination with *Salmonella* has declined since the implementation of HACCP from >20% to 7.3% (3), the incidence of human illnesses associated with *Salmonella* has remained relatively unchanged, at 15.9 cases/100,000 individuals (27). With the exception of additional in-plant intervention strategies (40), the next step to reduce *Salmonella* in

the finished product would be to reduce the number of *Salmonella*-infected birds entering the plant. This would require the identification of the most significant source of *Salmonella* for meat birds and implementing an effective, on-farm intervention strategy to prevent or reduce *Salmonella* colonization or shedding in chickens.

Vaccination may have a central role in the control of *Salmonella*, as it has the potential to reduce both the horizontal transmission of *Salmonella* among broiler breeder and broiler chickens and the vertical transmission of *Salmonella* from broiler breeder parents to broiler meat chickens (25). In the present work, we quantified the effect of vaccinating breeder flocks with live *S. Typhimurium* and killed *S. Berta* and *S. Kentucky* bacterin vaccines on *Salmonella* prevalence in broiler chickens. The adoption of a *Salmonella* vaccination program reduced the *Salmonella* prevalence in hens and their broiler chicken progeny. However, differences in prevalence were not apparent at the pullet farm level, where the breeder birds are raised to sexual maturity (4 to 5 months of age). *Salmonella* prevalence and load in broiler breeder chickens have been shown to be highest when hens begin their reproductive cycle or when they are subjected to stress (e.g., induced molting) (24, 30). Therefore, the effectiveness of vaccination is not likely to be apparent until birds reach sexual maturity and start their egg-laying cycle.

In this study, differences in *Salmonella* prevalence were not evident for samples collected from the broiler breeder farm environment. Davison et al. (15) previously reported a high percentage of environmental samples positive for *S. Enteritidis* despite the adoption of *S. Enteritidis* vaccination of commercial layer flocks. Despite the lack of a difference in *Salmonella* prevalence within the broiler breeder farm environment, the vaccinated hens themselves were less frequently colonized with *Salmonella*, as indicated by the significantly lower contamination of hen carcasses of company VAX. This finding is in agreement with previous work with the killed *S. Enteritidis* vaccine, which demonstrated a protective effect of vaccination on organ colonization by *Salmonella* but had a minimal effect on reducing fecal shedding of *Salmonella* (18, 19, 23, 24). Holt et al. (24) also reported little effect of an *S. Typhimurium*  $\Delta$ *cya*  $\Delta$ *crp* live vaccine on the transmission of *Salmonella* among *S. Enteritidis*-challenged and contact birds or reduction in the fecal shedding of *S. Enteritidis* following vaccination of layers.

Besides the protective effect of the *S. Typhimurium*  $\Delta$ *cya*  $\Delta$ *crp* live vaccine on reducing organ colonization by *Salmonella*, the live vaccine also decreases *Salmonella* colonization of eggs from vaccinated hens (22, 23) or at least reduced recovery from ovaries (24). The *S. Typhimurium*  $\Delta$ *cya*  $\Delta$ *crp* live vaccine also stimulates passive immunity, preventing the infection of newly hatched, highly susceptible birds with *Salmonella* (22). In this study, we observed a significantly lower *Salmonella* prevalence in newly hatched chicks from vaccinated hens. Moreover, we observed a significantly lower level of environmental contamination with *Salmonella* for poultry farms with placement of chicks from vaccinated breeder flocks, indicating that the vaccine reduced *Salmonella* prevalence or fecal shedding in the broiler progeny of vaccinated hens.

However, in the statistical models built, no farming practice had a better predictive value (more statistical significance) than the variable "company of origin" in determining the risk

of *Salmonella* contamination, suggesting that the use of vaccine may have been the most important determinant of *Salmonella* risk. For the company where a *Salmonella* vaccination program was not adopted, farmers appeared to be more aware of the need to adopt biosecurity measures (e.g., use of footbaths), and yet despite the implementation of these farming practices, the *Salmonella* prevalence was higher than that for the second poultry company that did not adopt these practices.

Live vaccines and killed vaccines, when used together, can effectively reduce the vertical and horizontal transmission of *Salmonella* to meat birds (6, 10, 11, 18–25, 29, 30, 31, 36, 38, 41). However, the eradication of *Salmonella* from the company was not observed. Other authors investigating the effects of *Salmonella* vaccination in laying hens (18), broiler breeder flocks (25), and their progeny (4) have also reported a failure of vaccine to eliminate *Salmonella* from poultry flocks. The live and killed *Salmonella* vaccines (*S. Berta*, *S. Kentucky*, and *S. Typhimurium*) used in this study were expected to provide protection against additional *Salmonella* serovars belonging to the same O serogroups (for example, *S. enterica* serovar Heidelberg) as the vaccines and cross-protection to other antigenically similar *Salmonella* O serogroups (serogroups A and E1). The vaccination would not provide immunity against antigenically unrelated *Salmonella* O serogroups (for example, serogroup C1). Therefore, the failure of the vaccines to eliminate *Salmonella* from vaccinated poultry flocks may reflect the prevalence and distribution of the other *Salmonella* O serogroups not covered by the vaccines. This, combined with the waning of the birds' immunity with age, may explain the failure of the vaccines to eradicate *Salmonella* from vaccinated breeder flocks and their broiler progeny. *Salmonella* vaccination should be used as one part of a comprehensive prevention program that includes other control measures and not as the sole intervention step for controlling *Salmonella* in poultry.

#### ACKNOWLEDGMENTS

Grants from USDA NRICGP 2005-01378, Epidemiological Approaches to Food Safety, and the U.S. Poultry and Egg Association supported this work.

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