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Resistance to Challenge of Breeders and Their Progeny with and Without Competitive Exclusion Treatment to *Salmonella* Vaccination Programs in Broiler Breeders

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Abstract: Resistance to *Salmonella* challenge of breeders under three vaccination programs and of their chicks with and without mucosal Competitive Exclusion (CE) (CHR Hansen) treatment was assessed. Vaccine treatments combined a live Aro-A *Salmonella* Typhimurium (ST) vaccine and an autogenous commercially prepared (Lohmann Animal Health) trivalent killed vaccine (serogroups B, C_2 and D_1). Treatments combined: 2 live and 2 killed doses or 3 live and 1 killed dose delivered at 1, 21, 77 and 126 d of age; or 2 killed doses delivered at 77 and 126 of age; and a non-vaccinated control (C). At 3, 6, 11, 17 and 22 wks of age, a portion of breeder pullets was removed and challenged *per os* with 10^7 cells of a 3-strain mixture of antibiotic-resistant salmonellae. Chicks from eggs laid at 29, 34 and 40 wks of age were randomly divided into two groups, one group received a CE treatment by oral gavage. Both groups were given 10^7 cells of a 2-strain mixture of antibiotic-resistant salmonellae and kept in isolation units for one and two wks. Ceca and Liver-Heart-Spleen (LHS) samples were cultured for each strain on BGS+antibiotic plates and colonies enumerated. Log_{10} data were analyzed under factorial designs. Breeder *Salmonella* counts showed significant reductions between (live) vaccinates and non-vaccinates at 3 (0.82 log) and 6 wks (0.85 log) challenges. By 11 wks, there were no differences in *Salmonella* levels between vaccinates and controls, indicating that 1-d and 3-wk live vaccine protection had diminished with time. All vaccination treatments reduced breeder cecal counts (1.15-1.30 log) by wk 22. Passive immunity from breeder vaccination treatments was not effective in diminishing chick cecal counts as shown by comparable susceptibility of chicks from vaccinated and control breeders, regardless of breeder age. Chick CE treatment consistently diminished cecal (1.41 log) and LHS (0.306 log) counts. These results show that live Aro-A ST vaccination decreases counts during the first 6 wks of age, as do all programs by 22 wks of age and that competitive exclusion is the most effective treatment in reducing hatchling *Salmonella* counts.

Key words: *Salmonella* challenge, vaccine, competitive exclusion, broiler breeders

Introduction

Exposure to *Salmonella* and subsequent enumeration of cecal and other organ samples, as well as measurements of indicators of humoral or cell-mediated immunity are the most common methods to assess chicken's resistance to *Salmonella* challenge. Early studies show the protective effect of autogenous preparations of live and killed vaccines to subsequent homologous serovar Typhimurium (ST) challenge, with best protection obtained when priming with live and boosting with killed oil-emulsion vaccine (Suphabphant *et al*., 1983). An attenuated ST strain by double deletion of genes coding for receptor protein of cAMP and adenylate cyclase (Δcrp and Δcya) was extensively studied. Application of the vaccine at 1 and 14 d of age (DOA) prevented colonization of the small intestine and reduced cecal and rectal counts when birds were challenged with a different ST strain at 21 or 28 DOA (Hassan and Curtiss, 1990). In a subsequent experiment, cecal colonization was prevented, with vaccine doses of 10^7 or 10^8 cfu mL^{-1} (Hassan *et al*., 1993). When protection to heterologous serovars (serogroups C1, C2, C3, D and E) was assessed, varying degrees of cross-protection to spleen, ovary, bursa, ileum, feces or cecal samples were observed, with a general tendency of better protection to (homologous) group B strains and limited protection to heterologous (C2, C3, E) strains. Even within serogroups, protection profiles varied: Challenge with a (group D) serovar Enteritidis (SE) strain showed bursal,

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fecal and cecal counts similar to controls, whereas fewer cfu mL^{-1} of fecal and cecal samples of birds challenged with serovar Panama (also group D) were observed (Hassan and Curtiss, 1994). A long-term study evaluating the protective effect of vaccination at 2 and 4 wks by challenge and culture with ST and SE at 3, 6, 9 and 12 months of age, showed that vaccination completely eliminated colonization of spleen, liver, ileum, ceca, ovary and reproductive tract samples, except for one positive SE isolation obtained from a magnum at 6 months of age (Hassan and Curtiss, 1997).

The use of *Salmonella* attenuated strains by deletion of the Aro-A gene (essential for the synthesis of chorismate) as potential vaccine candidates has also been studied. An AroA ST mutant initially reduced fecal excretion of an ST challenge strain on 4 DOA vaccinates, but the effect did not persist. Aro-A SE provided little protection either by oral or intramuscular administration at 20 and 22 wks of age (WOA), of birds challenged with SE at 24 wks. In contrast, a similarly-vaccinated group with the attenuated serovar Gallinarum mutant strain (R9) reduced liver, spleen, ovary and gut colonization by the challenge SE strain (Barrow *et al.*, 1990). A similar experiment showed reduced cfu mL^{-1} organ re-isolations from birds vaccinated with 9R but not with Aro-A SE and challenged with an SE phage type 4 strain (Barrow *et al.*, 1991). An Aro-A serovar Gallinarum (SG) was compared with the 9R ST vaccine and shown to protect if given intramuscularly (single dose at 2wks) but not orally against wild-type SG challenge. Mortality was reduced from 63 to 30% and from 30 to 12% for Aro-A and 9R vaccinated birds, respectively (Griffin and Barrow, 1993). In contrast to these investigations reporting limited protection by Aro-A mutants, other investigators found that Aro-A mutants provide adequate protection to challenge. A minimum of $10^{1.3}$ count reduction in feces and greater than 10^2 reductions in liver and cecal counts were obtained when birds were vaccinated with the Aro-A mutant at 1 and 14, or 1, 7, 14 and 21 d of age and challenged at 40 d of age (Cooper *et al.*, 1990). Further studies by these authors showed day of hatch (DOH) single-dose vaccination and challenge at 14 DOA using a seeder bird model protected the vaccinated group from colonization, but protection did not persist when challenged at 56 DOA. Birds vaccinated at 1 DOA and 2 wks, or at 1 DOA and 2, 16 and 18 wks and challenged at 23 wks showed similar reductions in organ counts, with greatest reductions shown by birds vaccinated four times (Cooper *et al.*, 1993). A 1 DOA and 16 wks vaccination program with 10^6 and 10^9 showed similar reductions in spleen, liver, ovary and cecal counts when birds challenged at 23 wks with SE. Only the higher vaccine dose reduced intestinal shedding. However, when birds were challenged with ST, organ counts were similar to controls, indicating limited protection to heterologous challenge (Cooper *et al.*, 1994). Intramuscular Aro-A ST vaccination at 3 DOA and intramuscular challenge with virulent ST at 7 DOA showed complete protection of vaccinates in contrast to controls, which did not survive challenge. The vaccine strain under a challenge model was shown to be shed for 5 d, but was eliminated by 14 d. A second experiment with oral DOH vaccination and challenge with varying virulent ST doses (10^4, 10^6, 10^8), showed that vaccinated birds stopped shedding by 35 DOA, whereas controls still had a 33% shedding frequency (Alderton *et al.*, 1991).

Studies reported in the literature describe delivery of live vaccines intramuscularly or by oral gavaging, but to our knowledge, no studies using modern commercial broiler breeders and administering vaccines by standard industry methods (aerosol at 1 DOA and in drinking water after 1 DOA) have been reported. Our previous work (Bailey *et al.*, 2007) profiled the humoral and gut mucosal IgA and IgG responses to vaccination programs using a licensed Aro-A ST vaccine alone or combined with an autogenous killed bacterin. In this study, the protective efficacies of these different vaccination programs against Salmonella in breeder hens were determined. In addition, the effects of passive immunity from the vaccinated hens on susceptibility of progeny chicks to *Salmonella* colonization was determined and compared to the efficacy of competitive exclusion treatment to protect the progeny chicks from *Salmonella* colonization.

Materials and Methods

Chickens, premises and vaccines: Chickens, premises and vaccines were fully described previously (Bailey *et al.*, 2007). Briefly, Cobb×Cobb broiler breeders from a commercial broiler supplier were placed at the University of Georgia's Poultry Science Research facilities. One-thousand chicks were randomly placed in disinfected premises, consisting of four environmentally-controlled industry-type rooms, with chain feeders and nipple drinkers, forced-air furnaces, negative ventilation systems and fresh pine shavings as litter material.

Vaccination treatments consisted of combinations of live Aro-A SE (Poulvac -ST®, Fort Dodge Animal Health Inc., Overland Park, KS) and a commercially-prepared oil-in-water emulsion containing serovars Heidelberg (group B), Kentucky (group C_2) and Berta (group D_1) (Lohmann Animal Health International, 1146 Airport Pkwy, Gainesville, GA 30501). The vaccines were administered in four different treatment combinations (Table 1): a non-vaccinated control, a two-live/two-killed (2L2K), a three-live/one-killed (3L1K) and a two-killed (2K) group. Vaccine delivery resembled commercial delivery practices, with live vaccine given as a coarse spray while inside chick boxes at day-of-hatch, or via drinking water at 21 or 77 DOA. Killed vaccines were delivered by neck subcutaneous injection at 77 and 126 DOA. Lighting and feeding programs followed commercial broiler breeder

Table 1: Recovery of *Salmonella* from ceca and internal organs of broiler breeders as influenced by different vaccine treatments

	Cecal Counts						
	2 Killed		2 Live-2 Killed		3 Live-1 Killed		Control
Week	Log cfu mL^{-1}	PF	Log cfu mL^{-1}	PF	Log cfu mL^{-1}	PF	Log cfu mL^{-1}
3	2.572^{a}		1.752^{b}	1.5	1.752^{b}	1.5	2.572^{a}
6	1.994^{a}		1.141^{b}	1.7	1.141^{b}	1.7	1.994^{a}
11	1.816^{a}		1.700^{a}	1.1	1.700^{a}	1.1	1.816^{a}
17	0.157^{b}	6.2	0.485^{ab}	2	0.675^{ab}	1.4	0.975^{a}
22	1.380^{b}	1.9	1.304^{b}	2	1.403^{b}	1.8	2.558^{a}
Liver-Heart-Spleen Counts							
3	0.359^{a}		0.278^{a}	1.3	0.278^{a}	1.3	0.360^{a}
6	0.088^{a}		0.044^{a}	2	0.044^{a}	2	0.088^{a}
11	0.219^{a}		0.000^{a}	Total	0.000^{a}	Total	0.220^{a}
17	0.000^{a}	Total	0.000^{a}	Total	0.000^{a}	Total	0.000^{a}
22	0.044^{a}	7.9	0.198^{a}	1.8	0.121^{a}	2.9	0.349^{a}

Vaccination Treatments: 2Killed = 2 killed vaccines given at 11 and 17 weeks of age; 2Live2Killed = 2 live vaccines given at days 1 and 21 and 2 killed vaccines given at weeks 11 and 17 of age; 3Live-1Killed = 3 live vaccines given at days 1, 21 and 77 and 1 killed vaccine given at 17 weeks of age; C = non-vaccinated controls. PF (Protection Factor) = Log cfu mL^{-1} of non-vaccinated controls / Log cfu mL^{-1} of vaccinated treatments. Means with different subscripts within rows are statistically significant ($p<0.05$).

husbandry practices. At 18 weeks of age WOA pullets were housed in four separate rooms (three treatment and one control) equipped with manually belt-conveyed nests, 2/3 slats and a central 1/3 mating/scratch area with softwood shavings and males introduced for mating.

Monitoring for environmental *Salmonella*: On arrival of chicks to the farm, chick box liners were cultured for *Salmonella* and 1 m^2 paper liners placed weekly under feeder troughs and cultured for *Salmonella* monitoring on d 7, 21, 42, 77, 98 and 119 of age. Chick box liners were cut, placed in a large stomacher bag with 250 mL of Buffered Peptone (BP) and contents manually mixed and incubated for 24 hr at 37°C. One mL of the BP was transferred to 9 mL of tetrathionate-brilliant green (TT) broth and incubated at 42°C for 24 hr. BG sulfa and Modified Lysine Iron Agar (MLIA) selective plates were then streaked for isolated colonies and incubated at 37°C for 24 hr.

Bacterial challenge strains and growth media: A mixture of 3 different antibiotic-resistant *Salmonella* serovars was used for all challenge studies: A rifampicin-resistant serovar Typhimurium (Rif-ST), a nalidixic acid-resistant serovar Enteritidis (Nal-SE) and an ampicillin-resistant serovar Thompson (Amp-STH), corresponding to serogroups B, D_1 and C_2, respectively. A pre-trial study with mixtures of 10^6-10^7 cfu/dose *per os* to 1 DOA broilers proved that all three isolates could be recovered one wk post-challenge and serovars segregated effectively on antibiotic-containing media. Media used for isolation was Difco® BG sulpha (BGS) agar (Becton Dickinson Diagnostics, Franklin Lakes, NJ) prepared in our laboratory with 200ppm of antibiotic (Rifampicin, Ampicillin or Nalidixic Acid) and 15ppm of Novobiocin added after autoclaving and just prior to plating.

Breeder challenge and bacterial enumeration: At 3, 6, 11, 17 and 22 wks of age,10 breeder pullets/treatment group were taken to the USDA's Poultry Microbiological Research Unit's research facilities in Watkinsville, GA, where they were placed in isolation units equipped with nipple drinkers, bell-type feeders and fresh pine shavings. About 10^7 cfu of a 24 hr culture of the three strain challenge *Salmonella* culture was administered by oral gavage to each breeder pullet/hen. One week post-challenge, the breeders were killed by cervical dislocation. Each breeder's left liver lobe, heart and spleen were pooled and placed on sterile stomacher bags with filter. Both ceca were removed and placed in a separate stomacher bag with filter. All samples were kept in ice until reaching the laboratory (less than 1h). Samples were weighed and peptone broth corresponding to three times sample weight added. Samples were stomached thoroughly and 500 µL of the suspension plated onto BGS plus corresponding antibiotic using a Spiraltech® plater (Spiraltech, Rockville, MD), incubated at 37°C for 24h and read using a Spiraltech® reader (Spiraltech, Rockville, MD). Two colonies from one fifth of all plates were serogrouped to confirm that colonies on each antibiotic-added plate corresponded to the expected serogroup.

Progeny challenge, competitive exclusion delivery and bacterial enumeration: Eggs from treatment breeders were collected at 29, 34 and 40 wks of breeder age and incubated. Immediately after hatch, 40 chicks per treatment were randomized into 4 subgroups, of which two subgroups were gavaged with 0.2 mL of Mucosal Starter Culture (MSC, CHR Hansen Inc., Milwaukee, WI), an undefined flora competitive exclusion culture, at a concentration of about 10 (10^9) porganisms/mL. Three to four h after MSC treatment, all chicks were challenged

Table 2: Recovery of different serotypes* of *Salmonella* from ceca and internal organs of broiler breeders

	Cecal Counts					
	Salmonella Enteritidis		*Salmonella* Thompson		*Salmonella* Typhimurium	
Week:	Log cfu mL^{-1}	% Comp.	Log cfu mL^{-1}	% Comp.	Log cfu mL^{-1}	%Comp.
3	2.112^{b}	10.7%	3.025^{a}	87.5%	1.348^{b}	1.8%
6	2.860^{a}	96.4%	0.454^{c}	0.4%	1.389^{b}	3.3%
11	2.841^{a}	87.8%	0.464^{c}	0.4%	1.969^{b}	11.8%
17	0.279^{b}	8.4%	1.286^{a}	85.3%	0.155^{b}	6.3%
22	1.691^{a}	33.1%	1.437^{b}	18.4%	1.857^{a}	48.5%
Liver-Heart-Spleen Counts						
3	0.749^{a}	68.4%	0.188^{ab}	18.8%	0.020^{c}	12.8%
6	0.198^{a}	44.1%	0.000^{a}	27.9%	0.000^{a}	27.9%
11	0.095^{a}	31.5%	0.000^{a}	25.3%	0.234^{a}	43.3%
17	0.000^{a}	---	0.000^{a}	---	0.000^{a}	---
22	0.066^{a}	25.2%	0.298^{a}	43.0%	0.169^{a}	31.9%

*Hens challenged with a suspension of approximately 10^{7} cells of *S.* Typhimurium, *S.* Enteritidis and *S.* Thompson, % Comp. = Percent serovar composition of total *Salmonella* isolated. Means with different subscripts within rows are statistically significant ($p<0.05$)

with a mixture of Rif-ST and Nal-SE containing at least 10^{7} cfu mL^{-1} of each strain. Eight chicks per subgroup were challenged and sampled as described earlier, one wk post-challenge for breeder age 29 wk and one and two wks post-challenge for breeder ages 34 and 40 wks. Bacterial enumeration for chick challenges was done following the swab-plate method of Bailey *et al.* (1988). Two colonies from one fifth of all plates were serogrouped to confirm that colonies on each antibiotic-added plate corresponded to the expected serogroup.

Statistical analysis: For each challenge event, data were transformed (Log_{10}) and analyzed under factorial designs. Main effects were Vaccination Treatment and Serovar for breeder challenges and Vaccination Treatment, Serovar and Competitive Exclusion Treatment for progeny challenges. Data were analyzed using SAS® (SAS Institute Inc., Carry, NC) software and mean differences discriminated using Student-Newman-Keuls' Multiple Range Test.

Results and Discussion

Environmental sampling: Environmental sampling yielded positive samples for *Salmonella* serovar Heidelberg on chick paper liners of female breeders on arrival, which was recovered at d 7, 21 and 42 but no longer at d 77, 98 and 119. This serovar was linked to a serovar commonly encountered at the hatchery and was apparently cleared by 42 DOA. No *Salmonella* was detected in the male breeder population. The *Salmonella* serovar Heidelberg found in environmental samples was present at very low counts and sensitive to low levels of all three antibiotics used during challenge trials. No growth of the field isolate was obtained in plates containing 100 ppm (1/2 the dose) of any of the three antibiotics used in the challenge model.

Breeder counts by vaccination treatment: All colonies that were serogrouped from antibiotic-added plates corresponded to expected serogroups, showing that reliable counts for each particular serovar in the multiple-strain model could be obtained from the same sample by plating on media containing the antibiotic to which each marker strain was resistant.

Day-of-hatch (day of hatch) vaccination with live Aro-A ST vaccine resulted in an average reduction of 0.82 log at 3 wks and a 0.85 log reduction at 6 wks of all serovar counts (Table 1). The live vaccine's protective effect waned by 11 wks. All vaccination treatments at wk 18 had numerically smaller counts compared to controls, but only the 2K treatment was statistically significant. Week 18 counts indicate that neither a vaccination program of two live (delivered at d 1 and wk 3) and one killed vaccine (delivered at wk 11), nor three live (delivered at d 1, wk 3 and wk 11), was better in reducing wk 18 cecal colonization than just a single killed vaccine delivered at wk 11. Contrary to our expectations, live vaccination at d 1 and wk 3 protected against early challenge, but had no booster effect measurable at wk 18. Challenge at wk 22 showed reduced counts for all vaccinates, indicating that all vaccination programs were equally efficient in reducing cecal colonization by this time. No differences for LHS counts due to vaccination treatments were observed.

Protection Factors (PF), defined as the ratio of *Salmonella* counts of treated groups to *Salmonella* counts of controls (Bailey *et al.*, 1988) and calculated for all treatments showed live vaccination conferred 1.5 and 1.7 PF for 3 and 6 wk counts. Values between 1.8 and 2.0 PF were obtained for 22 wk challenges. Protection factor values show that although reductions due to vaccination treatments were statistically significative, actual bacterial counts of controls versus vaccinates were between 1.5 and twice as great as non-vaccinated controls. These data show that vaccination helps in reducing overall counts but does not preclude *Salmonella* colonization.

Table 3: Recovery of *Salmonella* from the ceca of one or two-week old progeny of vaccinated broiler breeder hens

		Cecal Counts						
		2Killed		3Live-1Killed		2Live-2Killed		Control
Breeder Age (Wk)	Wks Post-Challenge*	Log cfu mL^{-1}	PF	Log cfu mL^{-1}	PF	Log cfu mL^{-1}	PF	Log cfu mL^{-1}
20	1	1.088^{a}	1.4	2.056^{a}	0.8	1.308^{a}	1.2	1.556^{a}
34	1	2.638^{a}	1.1	2.303^{a}	1.3	3.380^{a}	0.9	2.952^{a}
34	2	1.497^{a}	1.2	1.502^{a}	1.2	1.250^{a}	1.5	1.848^{a}
40	1	2.091^{a}	0.5	1.503^{ab}	0.7	1.145^{b}	1.0	1.108^{b}
40	2	1.289^{a}	0.8	1.086^{a}	1.0	0.998^{a}	1.1	1.081^{a}
Liver-Heart-Spleen Counts								
20	1	0.575^{a}	1.4	0.700^{a}	1.1	0.469^{a}	1.7	0.788^{a}
34	1	0.983^{a}	1.1	0.864^{a}	1.3	1.077^{a}	1.0	1.098^{a}
34	2	0.000^{b}	Total	0.211^{b}	2.6	0.127^{b}	4.3	0.548^{a}
40	1	0.352^{a}	0.7	0.352^{a}	0.7	0.386^{a}	0.7	0.258^{a}
40	2	0.539^{a}	1.0	0.534^{a}	1.0	0.455^{a}	1.2	0.539^{a}

*Chicks challenged with of approximately 10^{7} cells of *S.* Typhimurium and *S.* Enteritidis at one day-of-age and the, The ceca sampled for *Salmonella* one week after challenge, Vaccination Treatments: 2Killed = 2 killed vaccines given at 11 and 17 weeks of age; 2Live2Killed = 2 live vaccines given at days 1 and 21 and 2 killed vaccines given at weeks 11 and 17 of age; 3Live-1Killed = 3 live vaccines given at days 1, 21 and 77 and 1 killed, vaccine given at 17 weeks of age; C = non-vaccinated controls. PF (Protection Factor) = Log cfu mL^{-1} of non-vaccinated controls/Log of vaccinated treatments. Means with different subscripts within rows are statistically significant ($p<0.05$)

Table 4: *Salmonella* counts from the ceca and internal organs of one and two week old chicks as influenced by the administration of a competitive exclusion (Mucosal Starter Culture)* treatment on day-of-hatch

		Cecal Counts						
		Competitive Exclusion			Serovar			
		MSC		Control	*Salmonella* Enteritidis		*Salmonella* Typhimurium	
Breeder Age (Wk)	Wks Post-Challenge**	Log cfu mL^{-1}	PF	Log cfu mL^{-1}	Log cfu mL^{-1}%	Comp	Log cfu mL^{-1}	% Comp
20	1	0.686^{a}	3.3	2.231^{a}	0.503^{b}	1.2%	2.414^{a}	98.8%
34	1	2.067^{b}	1.7	3.569^{a}	1.239^{b}	0.1%	4.397^{a}	99.9%
34	2	0.957^{b}	2.2	2.091^{a}	0.245^{b}	0.3%	2.804^{a}	99.7%
40	1	0.702^{b}	3.2	2.221^{a}	0.295^{b}	0.5%	2.629^{a}	99.5%
40	2	0.435^{b}	4.1	1.792^{a}	0.085^{b}	0.9%	2.142^{a}	99.1%
Liver-Heart-Spleen Counts								
20	1	0.469^{b}	1.7	0.813^{a}	0.091^{b}	7.4%	1.191^{a}	92.6%
34	1	0.701^{b}	1.9	1.310^{a}	0.291^{b}	3.6%	1.720^{a}	96.4%
34	2	0.211^{a}	1.1	0.232^{a}	0.148^{a}	41.6%	0.295^{a}	58.4%
40	1	0.234^{b}	1.9	0.439^{a}	0.000^{b}	17.5%	0.673^{a}	82.5%
40	2	0.340^{b}	2.0	0.694^{a}	0.012^{b}	9.0%	1.014^{a}	91.0%

*MSC = Mucosal Starter Culture undefined flora competitive exclusion treatment; **Chicks challenged with of approximately 10^{7} cells of *S.* Typhimurium and *S.* Enteritidis at one day-of-age and the, The ceca sampled for *Salmonella* one week after challenge. % Comp. = Percent composition of serovar of all, *Salmonella* isolated. PF (Protection Factor) = Log cfu mL^{-1} of non-vaccinated controls/Log cfu mL^{-1} of vaccinated treatments. Means with different subscripts within rows are statistically significant ($p<0.05$)

Breeder counts by serovars: Counts obtained from composite LHS samples are an indicator of invasiveness. No consistency of relative serovar colonization through time was observed (Table 2). Serovar Thompson was more prevalent on 3 (87.5%) and 18 wk (85.3%) challenges; serovar Enteritidis was more prevalent on 6 (96.4%) and 10 wk (87.8%) challenges and serovar Thompson was slightly more prevalent on wk 22 (48.5%) challenge. Factors affecting intestinal microbial ecology (i.e. age of the birds, gut microflora composition), which are independent of vaccination treatments but vary through time, are probably responsible for this lack of serovar consistency between challenge events. However, in most challenge events, a particular serovar was more successful in establishing itself over the other two, as observed by the tendency for a particular serovar to be present at a higher concentration (% composition) at each challenge event. All LHS counts were substantially lower than corresponding cecal counts and in some cases no *Salmonella* was recovered from these samples.

Although counts were numerically somewhat higher in young birds (wk 3 challenge), no differences among treatments were observed.

Progeny counts by treatment: Progeny of vaccinated

breeders challenged at 1 day-of-age showed no effect from maternal antibody as measured by cecal counts, except for progeny from 40 wk-old breeders, sampled one wk post-challenge (Table 3). At this time, progeny from the 2K treatment had higher cecal counts than 2L2K (0.95 log) and Controls (0.91 log). This higher count was transient and counts were comparable to controls when progeny was sampled a wk later (2 wks post-challenge). Similarly, LHS samples showed no differences except between progeny of all vaccinated treatments and controls sampled 2 wks post-challenge, at 34 wks of breeder age. A mean 0.43 log reduction in vaccinates compared to controls was observed at this time. Serum maternal antibody passed through the yolk in these treatments was mainly IgG (Bailey *et al.*, 2007), with negligible IgA. Immunoglobulin G levels through time were consistently high and the slight differences in *Salmonella* counts observed at 34 wk cecal counts sampled 1 wk post-challenge and 34 wk LHS counts sampled 2 wk post-challenge cannot be directly related to differences in yolk IgG content. Immunoglobulin A passed through the egg (not measured) has been found to be more concentrated in the albumen (Kimijama *et al.*, 1990) and might play a greater role in initial protection against *Salmonella* challenge. The dynamics of albumen IgA as a response to vaccination of the dams may be different than the dynamics of IgG. Although we have not related albumen IgA concentrations to actual challenge, this is an area worth pursuing in future studies. The results seen in this study are in contrast to those observed by Hassan and Curtiss (1996) who found maternal antibodies to prevent colonization of chicks by homologous strains of *Salmonella* used to induce the immunological response. They also found that the maternal antibodies reduced the efficacy of the vaccination of progeny with live vaccines at one and three wks-of-age.

Progeny counts by serovar: Cecal and LHS counts were higher for the Rif-ST serovar on all progeny challenge events, except for LHS counts of progeny of 34 wk-old breeders, sampled 2 weeks post challenge (Table 4). This particular sample point showed the lowest overall *Salmonella* counts, which would explain the lack of differences between serovar counts.

Progeny counts by competitive exclusion: Delivery of MSC reduced *Salmonella* counts on all progeny challenge events, except for progeny of 29 wk-old breeders, where a numerical (not statistical) reduction was observed and overall counts were extremely small. Competitive exclusion was more effective than vaccination of breeders in reducing *Salmonella* hatchling colonization, as shown by a consistent reduction (1.35 to 1.55 logs) of cecal *Salmonella* counts of CE-treated chicks compared to controls (Table 4). Liver-Heart-Spleen counts also showed consistent reductions (0.02 to 0.35 logs), although these were lower in magnitude, as were LHS counts compared to cecal counts.

Throughout the 5 challenge events and for higher (cecal) counts, vaccination had a mean protection factor of 1, whereas competitive exclusion had a mean protection factor of 2.9. With lower (LHS) counts, differences in protection factors were much smaller, with a mean protection factor of 1.4 for vaccination treatments and 1.7 for competitive exclusion treatments. The higher protection factor values of CE-treated birds (Table 3) compared to vaccinated birds (Table 1) show that passive immunity obtained by the tested vaccination programs against *Salmonella* did not diminish counts as did the competitive exclusion treatment. These results highlight the importance of establishing beneficial gut microflora early in the life of the chick as an effective tool in curbing potential field challenges.

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Abbreviation Key: 2K = 2 killed vaccines; 2L2K = 2 live and 2 killed vaccines; 3L1K = 3 live and 1 killed vaccine; Amp-STH = Ampicillin-resistant *Salmonella Thompson*; BGS = brilliant green sulpha agar; CE = competitive exclusion; DOA = 1 d of age; LHS = liver-heart-spleen; MSC = mucosal starter culture; Nal-SE = nalidixic acid-resistant *Salmonella enteritidis*; PF = protection factor; Rif-ST = rifampicin-resistant *Salmonella typhimurium*; SE = *Salmonella enteritidis*; SG = *Salmonella gallinarum*; ST = *Salmonella typhimurium*; WOA = wk-of-age