ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 6 (6): 417-423, 2007
ISSN 1682-8356

Intestinal Humoral Immune Response and Resistance to Salmonella Challenge of Progeny from Breeders Vaccinated with Killed Antigen

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Abstract: *Salmonella* vaccination programs using killed bacterins in breeders and live auxotrophic-strain vaccines early in the life of their progeny have gained popularity in today's poultry industry. In this study we evaluated the intestinal humoral immune response to a live auxotrophic vaccine used on hatchlings with and without maternal antibody and related this response to challenge with a blend of two antibiotic-resistant *Salmonella* marker strains. Forty wk-old ISA Brown® (Institute de Selection Animale, France) breeders from a *Salmonella*-free flock were vaccinated twice at a three wk interval with commercially-prepared autogenous trivalent bacterin, serogroups B, C and D1 (Lohmann Animal Health International, Gainesville, GA), or a serovar Enteritidis bacterin (Fort Dodge Animal Health Inc, Overland Park, KS). Half of the progeny from these treatments (hatched from eggs laid 3 wks after second bacterin dose) were given a live *Salmonella* serovar Typhimurium (LiveST) mutant vaccine (Fort Dodge Animal Health Inc, Overland Park, KS), by coarse spray on arrival in the brooding premises. On days 3, 13 and 34, intestinal immunoglobulins (Ig) A and G were sampled and measured on enzyme-linked immunosorbent assay plates coated with *Salmonella* serovars Enteritidis (SELPS) or Typhimurium (STLPS) Lipopolysaccharide. On the same days, a second group of birds was challenged with a blend of antibiotic-resistant serovars Enteritidis and Typhimurium strains. Cecal and composite liver-heart-spleen samples obtained 7 days post-challenge were cultured and colonies enumerated. Maternal IgG observed up to 13 days had no effect on subsequent LiveST-stimulated antibody production. No protective effect of maternal antibody was demonstrated, except when combined with LiveST given to the progeny. Killed vaccines delivered to the breeders combined with a live vaccine delivered to the progeny resulted in reduced invasiveness after challenge, as shown by a reduction in liver-heart-spleen *Salmonella* counts. One dose of LiveST enhanced intestinal IgG [Optical Densities (OD) >0.576] up to 34 days when measured on STLPS, but only to 13 days when measured on SELPS, with titers decreasing with age. Increased IgA was observed only at 13 days. Three and 13 but not 34 days bacterial counts were decreased by the live ST vaccine treatment, for both cecal (1.05 and 1.09 $\log_{10}$) and liver-heart-spleen (0.32 and 0.06 $\log_{10}$) samples, indicating that a second dose might be necessary for prolonged protection. The protective effect of the live vaccine, but not of maternal IgG, leads us to hypothesize that protection might be due to stimulation of cell-mediated intestinal immunity and/or a competitive exclusion effect of the LiveST vaccine. Reduction but not elimination of *Salmonella* colonization by vaccination highlights the importance of vaccines as complementary tools and not substitutes of integral biosecurity programs to control *Salmonella* in poultry.

Key words: *Salmonella*, challenge, immune response, mucosal immunity, passive immunity, vaccine, breeders

Introduction

Although the cell-mediated and humoral intestinal immune responses are recognized as the primary mechanisms in defense against enteric bacterial pathogens, the intestinal immunoglobulin dynamics of progeny vaccinated with live auxotrophs shortly after hatch and its correlation to actual resistance to challenge in progeny has not been extensively studied. Vaccination of breeder flocks with autogenous *Salmonella* vaccines, as well as, vaccination of newly-hatched chicks with live vaccines has gained popularity in the poultry industry. However, there exists potential interference of maternal immunoglobulin in neutralizing live vaccines given early in life.

Early studies with turkey poults originating from breeders with a history of a *Salmonella* serovar Typhimurium field outbreak early in life and vaccinated multiple (4 to 6) times with an autogenous aluminum hydroxide-adjuvanted bacterin showed decreased overall poult mortality when challenged after hatch, compared with hatchlings from unvaccinated dams (McCapes *et al*., 1967). Turkey breeders vaccinated

twice with a *Salmonella* serovar Hadar bacterin at 41 and 45 wks and hatched from eggs laid at 51 wks, showed a decrease in the number of positive serovar Hadar isolates compared to turkey poults derived from non-vaccinated dams up to 39 days (Thain *et al.*, 1984). A reduction in *Salmonella* colonization of progeny from breeders vaccinated at 16 and 18 wks with a live Δcya Δcrp vaccine strain has been previously reported (Hassan and Curtiss, 1996). The authors also observed an interference of maternal antibody on early vaccination, since day-of-hatch delivery of a live serovar Typhimurium Δcya Δcrp vaccine strain to progeny with maternal antibody would clear the vaccine strain by two wks, compared to a persistence of the vaccine strain for at least 21 day in Specific Pathogen Free birds. In their study, maternally-derived immunity reduced efficacy of a 1 and 3 wk, but not a 2 and 4 wk vaccination program.

The present study was set to evaluate the effectiveness of early vaccination with a live *Salmonella* vaccine and in the presence of maternal antibody, in protecting hatchlings against *Salmonella* challenge at days 3, 13 and 34. Intestinal humoral (IgA and IgG) immune response and *Salmonella* counts seven days post-challenge were the parameters evaluated.

Materials and Methods

Breeders, vaccination and incubation: Forty wk-old ISA Brown® breeders (Institute de Selection Animale, France) in a small commercial operation were used as the parent stock. Breeders were housed in a 13.5 x 34 m open house with six 6.5 x 9.5 m compartments, three on each wing with a central corridor and transverse corridors separating each compartment. Each compartment housed approximately 380 female breeders. Litter material consisted of eucalyptus shavings and equipment consisted of manual bell feeders, automatic bell drinkers and metal nests with eucalyptus shavings as nest material. Nest shavings were changed on a bi-weekly basis, with 15g of paraformaldehyde added to each nest weekly. As part of the farm's established monitoring programs, routine bi-monthly environmental sampling for *Salmonella* during grow-out and production were carried out using drag swab, feed, litter and water samples. A 1% sample of the breeders were plate-agglutination tested with a polyvalent *Salmonella* antigen (Intervet International BV, Boxmeer, Holland) at start of production and 0.5-1% again after peak production. Any suspect birds were separated; sacrificed; and fecal and/or cecal samples taken which were cultured for *Salmonella*. Samples were pre-enriched in tetrathionate-brilliant green broth for 24hrs at 40°C before culturing on brilliant green sulpha agar.

Two of the six compartments were randomly chosen for vaccination treatments and a third chosen as a non-vaccinated control. Breeder vaccine treatments consisted of a monovalent serovar Enteritidis bacterin (SEBAC), POULVAC-SE (Fort Dodge Animal Health Inc., Overland Park, KS), or a commercially-prepared (Lohmann Animal Health International, Gainesville, GA) autogenous trivalent bacterin (TRIBAC) comprising serovars Heidelberg (serogroup B) Kentucky (serogroup C_2) and Berta (serogroup D_1). Breeders were vaccinated at 40 and 43 wks and progeny obtained from eggs laid at 46 wks.

Eggs were manually collected three to four times per day from outside the compartments, with nests placed on the perimeter of each compartment. Eggs were disinfected immediately after collection by submersion in a 0.5% solution of warm (37-45°C x 0.5-2min) Virkon-S (DuPont Animal Health Solutions, Wilmington, DE) disinfectant. Eggs were stored for 3-4 days at 14°C until incubation. The eggs were incubated in a single stage NSS-10 (Natureform Hatchery Systems Inc, Jacksonville, FL) incubator and hatched in separate trays, in an H-152 (Natureform Hatchery Systems Inc, Jacksonville, FL) hatcher along with eggs from the same breeder flock only. Samples of dead-in-shell embryos and chick box liners were also cultured for *Salmonella*. Chicks were vaccinated against Marek's disease (strains FC126/SB1/CVI988, Fort Dodge Animal Health Inc, Overland Park, KS) at hatch.

Chick treatments: On arrival to the farm, 396 chicks were randomized into 3 x 2 treatments (Breeder Bacterin x Live auxotroph vaccine), each treatment wingbanded with different colored bands, with a total of 33 chicks per treatment. While in the chick boxes, treatments receiving POULVAC-ST (Fort Dodge Animal Health Inc, Overland Park, KS)®, a live Aro-A auxotroph deletion mutant serovar Typhimurium strain (LiveST), were coarse-spray vaccinated and placed in the same room as treatments not receiving the LiveST, but in a different brooding pen. Each brooding pen was equipped with an infrared gas heater, two manual brooding feed pans and waterers, gradually replaced by one automatic bell drinker and manual tube feeder between 7 and 14 days. Rooms with brooding pens were previously disinfected by sublimation of 10 g of paraformaldehyde per m^3 of room volume 48 hrs prior to chick placement and with eucalyptus shavings and brooding equipment in place.

Intestinal immunoglobulin (Ig) sampling: On days 3, 13 and 34, 10 chicks per treatment were sampled for intestinal Ig. Chicks were removed from feed 16 hrs prior to sampling, euthanized and the small intestine excised at the ventriculo-duodenal and ileo-cecal junctions. Five ml of a lavage solution, consisting of 1M Tris/glycerine buffer and 0.25% Tween 20, as used by Holt *et al.* (1999) was flushed by inserting a feeding needle (Oxoid Inc, Ogdensburg, NY) through the ileal end and collecting flushed material through the duodenal end into 15 mL

centrifuge tubes. Samples were kept on ice until centrifuged at 2,500g for 10 min. The supernatant was frozen at -8°C until the enzyme-linked immunosorbent assay procedure was performed.

Elisa assays: The ELISA protocol was completed on each sample as previously described (Holt and Porter, 1993). Briefly, the assays were conducted using 96-well Immulon® plates (Dynex Technologies, Inc., Chantilly, VA) coated with Lipopolysaccharide from serovars Typhimurium (STLPS) or Enteritidis (SELPS) (Sigma Chemical Co., Saint Louis, MO), at a concentration of 10µL/ml, incubated overnight and blocked with a solution consisting of 1% bovine serum albumin, 1% phosphate buffered saline and 0.5ml/L Tween 20 (Sigma, Saint Louis MO). The blocking step solution was added to the plates for one hour. All plates were washed between steps twice with a 1% phosphate buffered saline plus 0.5ml/L Tween 20, for 2 to 3 minutes. Intestinal lavage samples were diluted 1:2 and added to the microplates together with controls and incubated for 90 minutes. Primary antibodies were mouse anti-chicken IgA or mouse anti-chicken IgG (Southern Biotech, Birmingham, AL) and diluted 1:1000. Primary antibodies were incubated for one hour. Secondary antibody was a goat anti-mouse heavy and light chain-specific IgG (Calbiochem, La Jolla, CA), used at a 1:2000 dilution and incubated for one hour. Para-nitro-phenyl phosphate chromogen (Sigma, Saint Louis, MO) at a 1mg/mL concentration diluted in diethanolamine (Sigma, Saint Louis, MO) was added, incubated for 20-30 minutes under dark conditions and plates read at 405nm.

Bacterial challenge: On the same days (3, 13 and 34), 10 chicks per treatment were challenged with a blend of 10^7-10^8 CFU/ml of a nalidixic acid resistant Serovar Enteritidis and a Rifampicin resistant serovar Typhimurium strains. Strains were grown 24hrs earlier on brilliant green sulpha agar with 200 ppm of corresponding antibiotic (rifampicin or nalidixic acid) and cells suspended in 0.85% saline. Cell suspensions were adjusted to 0.120 OD at 540nm and equal aliquots mixed prior to gavaging. The gavaging mix was plated and colonies counted to confirm that the mixed colony concentration was within the 10^8-10^9 cfu/ml range. Each chick was gavaged with 0.1ml of challenge mixture. Challenged chicks of all treatments were placed together in one brooding pen, in a separate room from non-challenged chicks. Seven days after challenge, six chicks per treatment were euthanized and sampled for bacterial enumeration. The left liver lobule, heart and spleen were pooled, weighed and placed in a stomacher filter bag (Fischer Scientific International Inc, Hampton, NH). Both ceca were removed and placed in a second stomacher bag with filter. All samples were kept in ice until processed (less than 2 h). Samples were weighed and peptone broth corresponding to 3 times sample weight added. Samples were stomached thoroughly and plated for enumeration as reported earlier (Bailey *et al.*, 1988), with some modifications. Briefly, four BGS plates per sample, two with rifampicin and two with nalidixic acid added at 200 ppm during preparation, were used. For each antibiotic-added plate, one plate was spread-plated with 100 µL of each stomached sample and a second with 100 µL of a 1:100 dilution of each sample. Hence, two plates with final dilutions of 10^{-1} and 10^{-3} per sample and per antibiotic were obtained. Plates were incubated at 37°C for 24 hrs and colonies counted. From each dilution (10^{-1} and 10^{-3}), plates with counts within or closest to a 30-300 colony per plate range were separated and enumerated for data analysis.

Statistical analysis: In order to contrast differences between treatments (vaccination regimens), ELISA data were analyzed under a completely randomized design and treatment differences contrasted using Duncan's Multiple Range Test. Additionally, to visualize the vaccination effects on ELISA profiles and *Salmonella* counts, transformed data (Log_{10}) were analyzed using the GLM procedure (SAS Institute, Cary, NY) under a factorial design (Breeder Killed Bacterin x Live Progeny Vaccine) and mean differences discriminated using Duncan's Multiple Range Test.

Results

Intestinal IgA profiles showed no measurable maternally-derived IgA, as revealed by similar OD of all treatments by day 3 (Fig. 1). Live vaccination elicited a small increase in intestinal IgA by day 13; the largest increase observed was on STLPS. The increased IgA was temporary (OD>0.65) and not present by day 34. In contrast, treatment IgG profiles shown (Fig. 2) for chicks from vaccinated breeders were higher than for chicks from non-vaccinated breeders at day 3 (OD>0.75). IgG titers decreased by day 13 and were no different from controls by day 34. Live vaccination increased IgG by day 13 (OD >0.75), but titers showed a declining trend by day 34. Titers were higher for live-vaccinated chicks by day 34 when measured on STLPS, but not on SELPS.

Analysis of the main effects (vaccines) on IgA and IgG (Table 1) show that LiveST caused a temporary small but significative rise in intestinal IgA by day 13, but this effect wasn't measurable by day 34. Although significative interactions for IgA when measured against STLPS on day 13 were observed, IgA titers between treatments not receiving LiveST were very similar to the control, with a slightly higher titer for non-vaccinated controls compared to breeder-vaccinated treatments. LiveST caused an increase in IgG for day 13 when measured on SELPS. This increased effect was greater

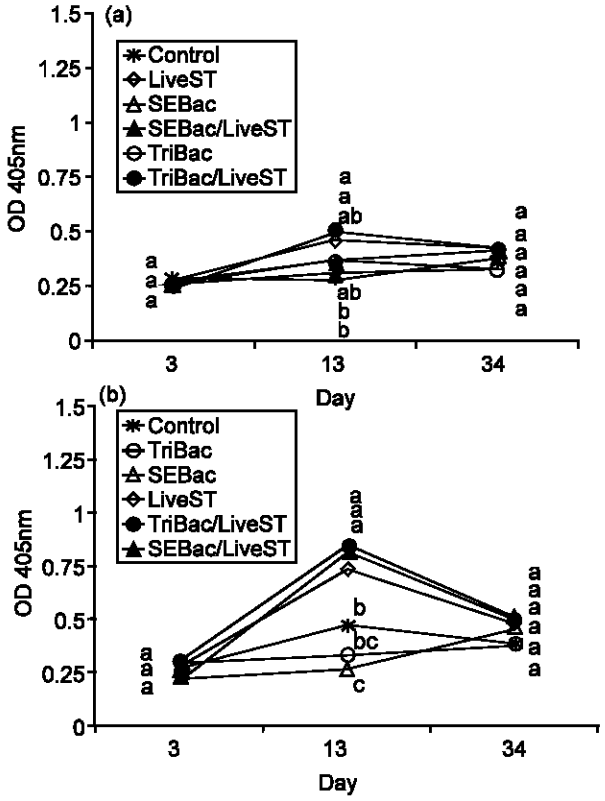

Fig. 1: Intestinal IgA optical densities of ELISAS with *Salmonella* serovars Enteritidis a) or Typhimurium b) lipopolysaccharide-coated plates; LiveST = live serovar Typhimurium vaccine; TriBac = trivalent serovars Heidelberg, Kentucky and Typhimurium bacterin; SEBac = serovar Enteritidis bacterin; Bacterins were delivered twice to breeders and live vaccine coarse-sprayed at day of hatch to their chicks. Intestinal lavage samples were taken on days 3, 13 and 34 of age. Means with different superscripts within each sampling day are statistically significant ($p<0.05$)

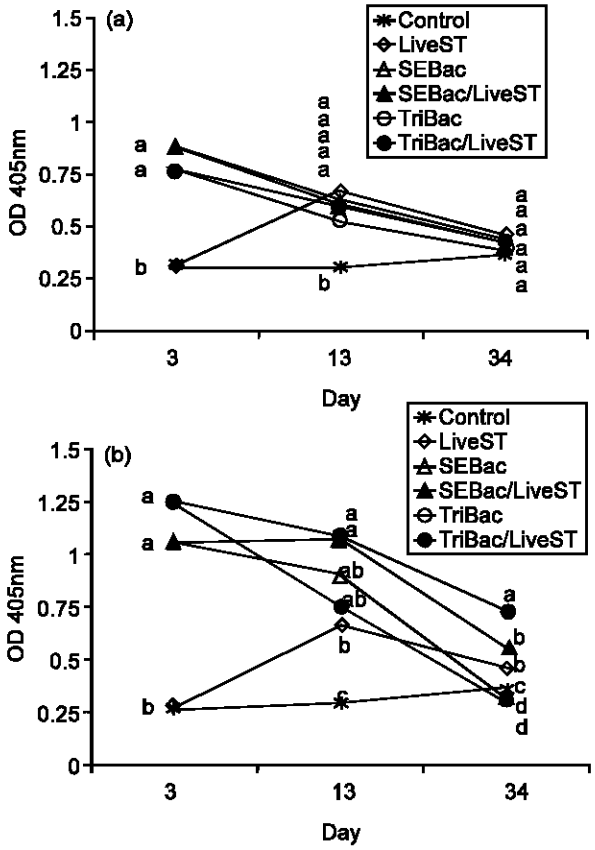

Fig. 2: Intestinal IgG optical densities of ELISAS with *Salmonella* serovars Enteritidis a) or Typhimurium b) lipopolysaccharide-coated plates; LiveST = live serovar Typhimurium vaccine; TriBac = trivalent serovars Heidelberg, Kentucky and Typhimurium bacterin; SEBac = serovar Enteritidis bacterin; Bacterins were delivered twice to breeders and live vaccine coarse-sprayed at day of hatch to their chicks. Intestinal lavage samples were taken on days 3, 13 and 34 of age. Means with different superscripts within each sampling day are statistically significant ($p<0.05$)

and lasted longer when measured on STLPS, as seen by increased titers for days 13 (mean OD = 0.934) and 34 (mean OD = 0.576). Maternally-derived IgG was higher for the TRIBAC treatment at day 3 when measured on SELPS and titers were higher from non-vaccinated controls throughout all sampling days when measured on STLPS. Although higher IgG was observed by day 34 for TRIBAC chicks, there was a consistent decrease in titers through time, as would be expected, with treatment means between TRIBAC and No-TRIBAC effects being very similar by day 34 (0.418 vs. 0.507 OD for No-TRIBAC and TRIBAC groups respectively). The same decrease in titers with time was observed with IgG measured on SELPS, but significant differences were observed only for days 3 and 13. A significative interaction for IgG measured on SELPS between LiveST and SEBAC shows a smaller rate of titer increase for SEBAC chicks that received LiveST than for non-SEBAC chicks that received LiveST for day 13 samples. Analysis of this interaction shows that non-SEBAC chicks receiving LiveST increased mean OD from 0.408 to 0.624, whereas SEBAC chicks receiving LiveST showed only a slight increase of mean OD, from 0.590 to 0.621. These data show that LiveST will result in similar end titers (OD of 0.621 and 0.624) regardless of the presence or not of maternally-derived antibody due to SEBAC treatment. Although an interaction for 34 day IgG on STLPS was detected, breakdown of data show OD

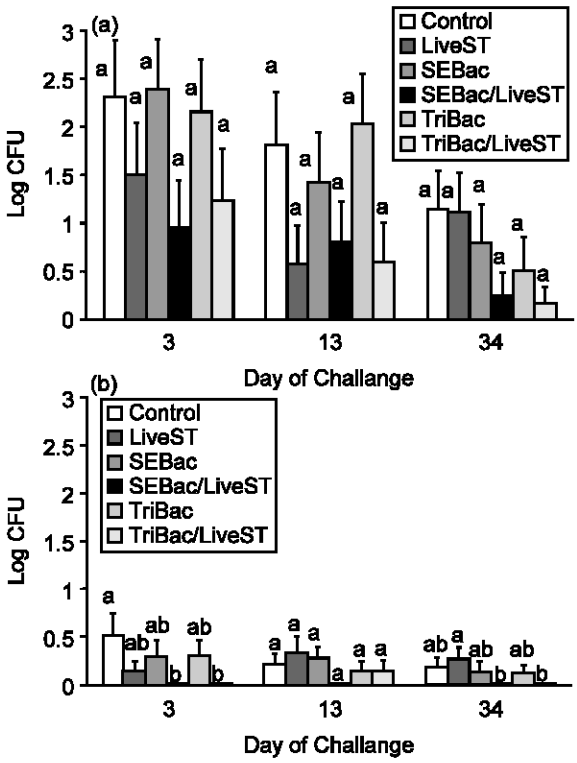

Fig. 3: Total *Salmonella* counts of a) cecal and b) pooled liver-heart-spleen samples; LiveST = live serovar Typhimurium vaccine; SEBac = serovar Enteritidis bacterin; SEBac/LiveST = Trivalent bacterin and live serovar typhimurium vaccine; TriBac = trivalent serovars Heidelberg, Kentucky and Typhimurium bacterin; SEBac/LiveST = Trivalent bacterin and live serovar typhimurium vaccine; Bacterins were delivered twice to breeders and live vaccine coarse-sprayed at day of age to their chicks. Chicks were challenged with mixed serovars Enteritidis and Typhimurium strains on days 3, 13 and 34 of age and bacterial counts assessed one week post-challenge. Means with different superscripts within each sampling day are statistically significant ($p<0.05$)

increases of 0.262 and 0.247 for no-SEBAC and SEBAC birds when receiving LiveST respectively. These values can be considered equivalent from a biological standpoint.

Total *Salmonella* counts showed no differences between treatments for cecal samples, but some differences were observed for liver-heart-spleen samples (Fig. 3). No *Salmonella* was recovered from liver-heart-spleen cultures from all treatments including breeder killed bacterin and live progeny vaccination, except for day 13 LiveST + SEBAC treatment. These differences were significant for days 3 and 34, but not for day 13. Although the LiveST treatment when contrasted to the other treatments did not show a decrease in liver-heart-spleen *Salmonella* counts, a factorial analysis of main effects (Table 2) reveals differences for LiveST for days 3 and 13, but not for day 34. Cecal sample data showed *Salmonella* count reductions of 1.05 and 1.09 $\log_{10}$ and liver-heart-spleen sample data showed reductions of 0.319 and 0.125 $\log_{10}$ for days 3 and 13 samples.

Discussion

The different IgG and IgA profiles at day 3 in chicks from vaccinated breeders show IgG is the most prevalent maternally-derived immunoglobulin type. Although we did not measure IgG and IgA levels in the breeders after vaccination, it appears that two doses of killed antigen delivered to the breeders induced IgG but not IgA passive immunity to the progeny. Maternal IgG is deposited in the egg yolk and IgA is deposited in the amniotic fluid, which is swallowed by the embryo prior to hatching (Rose *et al.*, 1974). Passive yolk-derived IgG is parenterally transferred to the embryo through the vitelline vessels, or enters the intestinal lumen via the omphaloenteric duct and can be detected as early as 3 days prior to hatch (Kimijama *et al.*, 1990; Magyar *et al.*, 1997). Maternally derived IgG in serum is reported to be highest immediately after hatch and decreases after 2 to 5 wks (Kowalczyyk *et al.*, 1985). In our study, intestinal IgG followed a similar trend. We were unable to clearly demonstrate interference of maternally-derived immunoglobulins on the LiveST vaccination treatment's ability to elicit increased IgG and IgA titers in the offspring of the vaccinated breeders. However, treatments combining breeder vaccination and progeny LiveST were the most effective in eliciting higher IgG titers for days 13 and 34 and IgA titers for day 13. This effect is more evident when reviewing the ELISA data using STLPS antigen. Higher titers on STLPS would be expected when the birds were vaccinated with a serogroup B (LiveST). LiveST is a serovar Typhimurium strain and serovar Heidelberg, contained in the TRIBAC bacterin, is a group B serovar, therefore they share common somatic antigens 4, 12 and possibly 5 (Anonymous, 1998). We observed marginally lower IgG titers for the SEBAC treatment when measured on STLPS and marginally higher titers when measured on SELPS. In general, ELISAS using LPS as capture antigen tend to be more sensitive as somatic antigen homology with the challenge/vaccine strain eliciting the immune response increases.

IgA priming by the liveST was shorter lived than IgG. Previous research has shown serum IgA and IgG titers after six wk-old prime-infection with an invasive serovar Typhimurium strain to persist up to 10 wks post challenge (Beal *et al.*, 2004). However, immunoglobulin responses on younger birds elicited by LiveST appear to be shorter lasting. This may be explained in part by the

Table 1: IgA and IgG profiles[A] as affected by a day-of-hatch live *Salmonella* serovar Typhimurium vaccine, or maternally-derived from breeders vaccinated with a killed *Salmonella* serovar Enteritidis bacterin, or a trivalent (*Salmonella* serovars Heidelberg, Kentucky and Berta) bacterin

	IgA Optical Density on Serovar Enteritidis Lipopolysaccharide						Interaction P Value	
Day of Age	No LiveST	LiveST	No TriBac	TriBac	No SEBac	SEBac	LiveSTxTriBac	LiveSTxSEBac
3	0.268[a]	0.268[a]	0.278[a]	0.249[a]	0.262[a]	0.280[a]	-	-
13	0.318[a]	0.439[b]	0.355[a]	0.426[a]	0.399[a]	0.337[a]	0.566	0.140
34	0.347[a]	0.418[a]	0.387[a]	0.374[a]	0.384[a]	0.379[a]	0.539	0.646
3	0.261[a]	0.261[a]	0.244[a]	0.294[a]	0.281[a]	0.220[a]	-	-
13	0.351[a]	0.794[b]	0.567[a]	0.582[a]	0.591[a]	0.220[a]	0.029*	0.012*
34	0.401[a]	0.488[a]	0.451[a]	0.430[a]	0.427[a]	0.220[a]	0.905	0.798
	IgG Optical Density on Serovar Enteritidis Lipopolysaccharide						Interaction P Value	
Day of Age	No LiveST	LiveST	No TriBac	TriBac	No SEBac	SEBac	LiveSTxTriBac	LiveSTxSEBac
3	0.646[a]	0.646[a]	0.590[a]	0.758[b]	0.531[a]	0.876[b]	-	-
13	0.469[a]	0.623[b]	0.542[a]	0.554[a]	0.516[a]	0.606[a]	0.065	0.041*
34	0.375[a]	0.434[a]	0.404[a]	0.405[a]	0.406[a]	0.402[a]	0.550	0.746
3	0.852[a]	0.852[a]	0.660[a]	1.237[b]	0.752[a]	1.053[b]	-	-
13	0.645[a]	0.934[b]	0.729[a]	0.911[b]	0.694[a]	0.981[b]	0.844	0.285
34	0.319[a]	0.576[b]	0.418[a]	0.507[b]	0.457[a]	0.428[a]	0.057	<.0001**

[A]Optical Densities at 405nm; LiveST = Live *Salmonella* serovar Typhimurium vaccine; TriBac = Trivalent *Salmonella* serovars Heidelberg, Kentucky and Berta bacterin; SEBac = *Salmonella* serovar Enteritidis bacterin; [ab]Means with different superscripts within each main effect are statistically significant ($p<0.05$); Significant interactions are depicted at 0.05 (*) and 0.01 (**) p values.

Table 2: Cecal and liver-heart-spleen total *Salmonella* counts as affected by day-of-hatch live *Salmonella* serovar Typhimurium vaccine, or by dam vaccination with a killed *Salmonella* serovar Enteritidis bacterin, or a trivalent (*Salmonella* serovars Heidelberg, Kentucky and Berta) bacterin

	Liver-Heart-Spleen Total *Salmonella* Counts[A]						Interaction P Value	
Day of Age	No LiveST	LiveST	No TriBac	TriBac	No SEBac	SEBac	LiveSTxTriBac	LiveSTxSEBac
3	0.367[a]	0.048[b]	0.236[a]	0.150[a]	0.237[a]	0.149[a]	0.845	0.840
13	0.215[a]	0.160[b]	0.206[a]	0.150[a]	0.212[a]	0.139[a]	0.639	0.092
34	0.151[a]	0.090[a]	0.150[a]	0.062[a]	0.145[a]	0.072[a]	0.209	0.167
	Cecal Total *Salmonella* Counts[A]						Interaction P Value	
Day of Age	No LiveST	LiveST	No TriBac	TriBac	No SEBac	SEBac	LiveSTxTriBac	LiveSTxSEBac
3	2.284[a]	1.230[b]	1.788[a]	1.696[a]	1.799[a]	1.673[a]	0.918	0.553
13	1.749[a]	0.659[b]	1.151[a]	1.310[a]	1.249[a]	1.115[a]	0.830	0.525
34	0.806[a]	0.509[a]	0.817[a]	0.337[a]	0.731[a]	0.510[a]	0.636	0.448

[A]Log10 CFU/mL; LiveST = live *Salmonella* serovar Typhimurium vaccine; TriBac = *Salmonella* trivalent serovars Heidelberg, Kentucky and Berta bacterin; SEBac = *Salmonella* serovar Enteritidis bacterin; [ab]Means with different superscripts within each main effect are statistically significant ($p<0.05$)

lack of invasive nature of the auxotroph mutant, as well as, by the hypo-responsiveness of young birds to early infection (Holt *et al.*, 1999). Although LiveST and TRIBAC yielded higher IgG responses throughout 34 days (Table 1), IgG titers consistently decreased after day 13, approaching control values by day 34. A second dose of LiveST is probably necessary if longer-lasting immunoglobulin titers are desired, such as for birds with a longer lifespan than commercial broilers (*i.e.* breeders and layers).

The lack of treatment differences for total *Salmonella* cecal counts despite differences in immunoglobulin profiles (Fig. 1 and 2) show that increased intestinal IgG and IgA were not sufficient to effectively reduce *Salmonella* incidence. It is interesting to note, however, that combining maternal immunity with LiveST vaccination diminished invasiveness of challenge strains at all sampling events, except for day 13 SEBacxLiveST treated birds (Fig. 3b). Innate and cell-mediated immunity as well as adequate humoral responses are important components of immunity against bacterial pathogens. Ontogenic studies of the gut-associated lymphoid tissue have shown the presence of IgG and IgA B cells as early as 5 days post-hatch and IgA and IgG plasma cells by 14 days post-hatch in the intestinal lamina propia (Jeurissen *et al.*, 1989). However, a recent study using chicken Interferon γ mRNA as a marker of T-cell effector functionality showed reduced Interferon γ expression of intestinal T cells, leading the authors to conclude that gut resident T cells are functionally immature during the first 2 weeks of life (Bar-Shira *et al.*, 2003). Similarly, suboptimal functional activity of the heterophil in young chicks as measured by decreased phagocytic indices on 1 and 4 day-old chicks compared to 7 day-old chicks show that innate immunity also undergoes maturation with time (Wells *et al.*, 1998).

The beneficial effect of LiveST in young chicks may be explained not only by an increase in intestinal

immunoglobulins, but by colonization and hence competitive exclusion effect from the vaccine strain. The potential competitive exclusion effect of vaccine strains against *Salmonella* challenge has been previously hypothesized (Berchieri and Barrow, 1990). Studies combining day-of-hatch vaccine or homologous strain delivery followed shortly (as early as 2 days post vaccination) by challenge show a decrease in colonization of birds previously vaccinated/colonized (Methner *et al.*, 1997). The short time lapse between vaccination and challenge probably is not enough for an immune response, but a decrease in colonization apparently points towards an initial competitive-exclusion effect from the vaccine, which is complemented by immune-priming with time. Although LiveST vaccination decreases overall bacterial load, *Salmonella* presence in the ceca in considerable numbers highlights the importance of vaccine programs in breeders and newly hatched chicks as a complementary tool in controlling *Salmonella* in poultry. However, this work shows that by no means may vaccination programs be a substitute for implementation of adequate biosecurity programs throughout breeding, production and processing.

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Abbreviations: IgA = Immunoglobulin A; IgG = Immunoglobulin G; LiveST = Live Aro-A Mutant *Salmonella* serovar Typhimurium vaccine; SEBAC = *Salmonella* serovar Enteritidis bacterin; SELPS = *Salmonella* serovar Enteritidis lipopolysaccharide; STLPS = *Salmonella* serovar Typhimurium lipopolysaccharide; TRIBAC = trivalent autogenous bacterin, serogroups B, C_2 and D_1; OD = Optical Density; wk = week