Live salmonella typhimurium vaccination of broilers results in cross-protection against other salmonella serotypes at different ages of exposure

Manuel Da Costa, Kalen Cookson, Jean Sander, Tak Niino, Andy McRee, Jon Schaeffer, and John Dickson

Zoetis, U.S. Poultry

Introduction

The Food Safety and Inspection Service (FSIS) announced the implementation of new Salmonella spp. performance standards for poultry processing plants in May 14th, 2010. Since then, the poultry industry has been adapting and testing several strategies that aim to reduce the levels of Salmonella spp. Live-side interventions that help reduce both the prevalence and the load of Salmonella spp. are a key component of successful salmonella control programs. Vaccination of broilers with live Salmonella typhimurium vaccines is a strategy that has gained ample traction and success in the past few years. The objective of the following four studies was to evaluate the effect of a live Salmonella typhimurium vaccine [Poulvac® ST (PVST)] on cross-protection against three Salmonella serotype challenges given at early and late ages to broiler chickens.

Materials and Methods

Four broiler studies were conducted to evaluate the cross-protective effect of PVST against four salmonella serotypes when exposed to either early or late challenges. For the early challenge studies, Salmonella spp. load (enumeration) was determined by most probable number (MPN) methodology in liver/spleen and cecae samples. For the late challenge studies, Salmonella spp. prevalence was determined in liver/spleen and load in cecae samples. The four studies had the following designs:

Early challenge studies:

Study 1 (Salmonella enteritidis): 150 broiler chicks were placed into 6 different isolators (25 per) at day of age. Three isolators received birds vaccinated with PVST by spray at hatch and boosted by oral gavage at 14 days. Birds from the three remaining isolators were not vaccinated (control group). At 4 days of age, all birds were challenged by oral gavage with Salmonella enteritidis at a target dose of $10^4$ CFU per bird. At 21 days (17 days post-challenge), all birds were terminated and sampled.

Study 2 (Salmonella kentucky): 110 broiler chicks were placed in an isolation room into two different pens (55 birds per pen) at day of age. One of the pens received birds vaccinated with PVST by spray at hatch whereas the other received birds not vaccinated (control group). At 5 days of age, all birds were commingled into one big pen and challenged by oral gavage with Salmonella kentucky at a target dose of $10^6$ CFU per bird. At 14 days (9 days post-challenge), all birds were terminated and sampled.

Late challenge studies:

Study 3 (Salmonella heidelberg): 270 broiler chicks were divided into three isolation rooms with two different pens each (45 birds per pen) at day of age. In each room, one of the pens received birds vaccinated with PVST by spray at hatch and boosted by gavage at 14 days whereas the other pen received birds not vaccinated (control group). At 35 days of age, all birds were challenged by oral gavage with Salmonella heidelberg (SH) at a target dose of $10^9$ CFU per bird. At 49 days (14 days post-challenge), all birds were terminated and sampled.

Study 4 (Salmonella kentucky and S. infantis): 128 broiler chicks were placed in an isolation room into two separate pens (64 birds per pen) at day of age. One of the pens received birds vaccinated with PVST by spray at hatch and boosted by gavage at 14 days whereas the other pen received birds not vaccinated (control group). At day 34, half of the birds from each pen were transferred into another isolation room and comingled in one big pen (64 birds total with 32 birds per treatment) and were challenged with Salmonella kentucky (SK) at a target dose of $10^6$ CFU per bird. The remainder birds (64 birds total with 32 birds per treatment) that stayed in the room were comingled and challenged with Salmonella infantis at a target dose of $10^6$ CFU per bird. At 46 days (12 days post-challenge), all birds were terminated and sampled.
All statistical analyses were conducted at a 0.05 level of significance ($P < 0.10$ considered as a trend) using
two-sided tests. Studies 1 and 3 were conducted with progeny from breeders vaccinated with a killed *Salmonella
enteritidis* vaccine, whereas study 2 and 4 had progeny from breeders not vaccinated for Salmonella.

**Results**

The early and late challenge results are presented in tables 1 and 2 respectively.

**Study 1:** upon early *Salmonella enteritidis* challenge, the load of *Salmonella spp.* in liver/spleen samples
tended ($P = 0.077$) to be lowered (-0.57 log) by PVST vaccination. In addition, cecal load was 0.92 log numerically
($P = 0.234$) lower in PVST vaccinated birds.

**Study 2:** on the early *Salmonella kentucky* challenge, the cecal load of *Salmonella spp.* tended ($P = 0.072$)
to be lower in PVST vaccinated birds. There was no statistical difference ($P=0.489$) in the liver/spleen enumeration
results.

**Study 3:** upon late *Salmonella heidelberg* challenge, both liver/spleen prevalence ($P = 0.043$) and cecal load
($P = 0.003$) were significantly lower in samples obtained from vaccinated birds. When compared with the controls,
the vaccinated birds had Salmonella prevalence and load reduced by 75.00% and 1.54 log, respectively.

**Study 4:** upon late *Salmonella kentucky* challenge, both the liver/spleen prevalence ($P = 0.001$) and cecae
load ($P=0.027$) were reduced by PVST. Vaccination was associated with a 75.78% reduction of liver/spleen
prevalence and 0.49 log lower cecal load. For the late *Salmonella infantis*, cecal *Salmonella spp.* enumeration ($P =
0.064$) and prevalence ($P=0.196$) by MPN (limit of detection ≥ 4 colony forming units) tended to be lowered by
PVST vaccination. The PVST vaccinated birds had 75.73% less positive cecae than birds not vaccinated. There were
no statistically significant differences ($P = 1.000$) in liver/spleen prevalence.

**Discussion**

After spray vaccination of day old birds with live *Salmonella typhimurium* vaccines, the bacteria are
ingested and rapidly colonize the gut epithelial cells. At a first stage, populating the gut mucosa with the vaccine
strain prevents the attachment and colonization of wild salmonella types that might be present at the hatchery,
transport trucks, or brooding areas. Simultaneously, innate and humoral (especially IgA – mucosal immunity)
responses are triggered in order to protect and mount an immune response to the vaccine strain. Knowing that broiler
chicks are more susceptible to Salmonella colonization at day of age than at later stages of life (2), it is crucial that
the Salmonella vaccination takes place right after hatch allowing not only a better take and more efficient
vaccination, but also a better competitive exclusion.

One of the goals of the studies presented herein was to evaluate the capability of a live *Salmonella
typhimurium* vaccine to respond to early Salmonella challenges where little time was allowed to construct a full
immune response. Study 1 showed that PVST was able to reduce the number of *Salmonella enteritidis* in the
liver/spleen when birds were challenged at 4 days of age and sampled at day 21. This finding indicates that even
though there was a high load of Salmonella present in the cecae, the birds that were vaccinated had less Salmonella
colonizing these organs. In study 2, PVST reduced the load of *Salmonella kentucky* in the cecae 9 days post
challenge. The liver/spleen salmonella enumeration results were low overall suggesting that there was a poor organ
colonization. The results of these two studies show the value of early vaccination with PVST on cross-protecting
against salmonella from groups D and C.

Studies 3 and 4 with the associated late salmonella challenges, were intended to evaluate the cross
protective effects of live Salmonella vaccination close to processing age in broilers. Overall, PVST effectively
reduced the colonization of the three serotypes tested. Both *Salmonella heidelberg* and *Salmonella kentucky* had
significant reductions in prevalence and load in the liver/spleen and cecae, respectively. The *Salmonella infantis*
challenge had a lower take compared with the other two serotypes which could have limited the ability to detect
treatment effects. Nevertheless, PVST vaccination tended to reduce the *Salmonella infantis* load and prevalence in
the cecae.

In a comprehensive review on host immunity to Salmonella, Lillehoj et. al (1) emphasize that each serotype
triggers different cellular and humoral immune responses and that, despite these differences, Salmonella vaccines
have shown encouraging results in controlling Salmonella infection. The data presented herein reiterate the
observations by Lillehoj et. al (1) since the live *Salmonella typhimurium* vaccine was shown to cross-protect against
three other serotypes. In conclusion, the administration of live salmonella vaccination in broilers was shown to be an
effective live-side intervention against early and late challenges of salmonellas belonging to the three most common salmonella O-groups (B, C, and D).

Table 1. Effects of PVST vaccination against *Salmonella enteritidis* and *Salmonella kentucky* challenges on liver/spleen and cecae *Salmonella* spp. load.

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<th>Early challenge</th>
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<td></td>
<td>Liver/spleen enumeration (Log CFU/ml)</td>
<td>Liver/spleen enumeration (Log CFU/ml)</td>
<td>Cecae enumeration (Log CFU/ml)</td>
<td>Cecae enumeration (Log CFU/ml)</td>
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<td>Control</td>
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<td>PVST</td>
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<td>0.37</td>
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<td>0.077</td>
<td>0.489</td>
<td>0.234</td>
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Table 2. Effects of PVST vaccination against *Salmonella heidelberg*, *Salmonella kentucky* and *Salmonella infantis* challenges on liver/spleen prevalence and cecae load of *Salmonella* spp.

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<td><em>Salmonella infantis</em></td>
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<td></td>
<td>Liver/spleen prevalence (%)</td>
<td>Liver/spleen prevalence (%)</td>
<td>Liver/spleen prevalence (%)</td>
<td>Cecae enumeration (Log CFU/ml)</td>
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<td>0.001</td>
<td>1.000</td>
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<td>0.027</td>
<td>0.064</td>
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References