

# Efficacy of a *Salmonella* Typhimurium and *Salmonella* Choleraesuis experimental combination vaccine against *S. Typhimurium* challenge in growing pigs.

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*Salmonella* Typhimurium (ST) and Choleraesuis (SC) are primary pathogens in swine. The objective of this study was to evaluate the efficacy of an experimental vaccine containing two avirulent live cultures (ALC) of ST and SC administered in pigs to aid in the prevention of salmonellosis caused by a *Salmonella* Typhimurium (ST) challenge. Pigs (n=48) were randomly assigned to one of two treatment groups: 1) placebo or 2) vaccinated. Pigs were administered either placebo or vaccine at 16-17 days of age through the drinking water with individual cup waterers. At four weeks post-vaccination, placebo-treated and vaccinated pigs were commingled and intranasally challenged with >10 logs of a virulent ST. Clinical observations and fecal shedding of ST were assessed daily for ten days post-challenge. Surviving pigs were then euthanized, and intestines were scored for pathological lesions consistent with *Salmonella* infection. Pigs were considered affected with salmonellosis if the cecum, spiral colon, mesenteric or ileocecal lymph nodes had a non-zero macroscopic lesion score or if the pig died due to challenge. Prevented fraction (PF) analysis was utilized to evaluate prevention of salmonellosis, and mitigated fraction (MF) analysis was used to evaluate reduction of diarrhea and ST shedding. Vaccination reduced salmonellosis with a PF of 53.3% (95% CI; 7.7, 80.7). Vaccination reduced the duration of diarrhea post-challenge with a MF of 75% (95% CI; 47.8, 94.7). Although all pigs shed ST post-challenge, vaccination reduced the duration of shedding with a MF of 45% (95% CI; 20.8, 72.2). In addition, body weight was measured to assess the impact of challenge on weight gain. Vaccination resulted in 2.42 kg heavier pig during the post-challenge period (P=0.0006). The data supports vaccination with a single dose of this ALC vaccine containing *Salmonella* Typhimurium and *Salmonella* Choleraesuis administered through the drinking water aids in the prevention of salmonellosis.

## Introduction

*Salmonella enterica* serovar Typhimurium (ST) and *Salmonella* Choleraesuis (SC) are identified as primary pathogens in swine. ST is a primary cause of enteritis and subclinical production losses in growing or finishing swine and contributes to environmental and carcass contamination. SC is a primary cause of septicemia, pneumonia and/or enterocolitis in growing pigs. The objective of this vaccination-challenge study was to evaluate the efficacy of an experimental combination vaccine composed of two avirulent live bacterial cultures (ALC) of ST and SC administered once to two-week-old pigs via the drinking water as an aid in the prevention of salmonellosis caused by a virulent *Salmonella* Typhimurium challenge.

## Materials and Methods

Sixty conventional, two-week old pigs originated from a herd without a history of clinical salmonellosis and without historical use of *Salmonella* vaccines were utilized in this study. Pigs were sourced from twelve litters and randomly assigned to each treatment group accordingly: one pig to the strict control group, and two pigs for each of the two treatment group (placebo and vaccine). A description of the treatment groups is shown in Table 1. Baseline fecal samples and blood samples were collected for *Salmonella* culture and *Salmonella* serology (tested by IDEXX *Salmonella* ELISA). Fecal samples were required to be negative by enrichment culture for pigs to be included in the study. Weights were collected on D-1, D27 and D38. Pigs were individually offered the ST/SC vaccine or placebo in 60mL of drinking water per pig at 16-17 days of age (D0). During the vaccination phase each of the three groups were housed in separate rooms to avoid unintentional exposure to vaccine that may be shed in the feces by the vaccinated pigs. The twelve strict control pigs were included as cohorts to the study animals in order to monitor biosecurity and were not included in statistical comparisons. The strict control animals were necropsied on D27 of the study to evaluate overall health. Bacterial culture of the organs determined no prior *Salmonella* colonization. Prior to challenge, the remaining pigs were re-penned and comingled so that vaccinated and placebo-treated pigs were both represented within rooms.

On D28, the vaccinated and placebo treated pigs were challenged intranasally with 2mL of a virulent ST (>10<sup>10</sup> CFU per dose)(1). For ten days, pigs were observed daily for clinical signs of salmonellosis. Feces were scored for the presence, severity, and duration of diarrhea; duration was assessed by the number of days elapsed from the first to the last positive observation. Necropsies were conducted ten days post-challenge (D38) or earlier in the event of severe disease and mortality. Blood was collected for serology and fecal samples were collected throughout the study to monitor shedding via direct and modified enrichment cultures (3). A pig was considered to be shedding ST if a fecal sample was positive for non-vaccine serogroup B *Salmonella* post-challenge. Direct culturing was performed on fecal samples post-challenge and organs at necrop-

sy with an approximate sensitivity of 150 colony forming units per gram (cfu/gram). Dilutions of fecal material were done for quantitative shedding. To determine an average cfu/gram of feces for the group, enrichment positive cultures were assigned the values of “1” and the lower limit of detection for direct cultures was determined to be 150 cfu/gram. Body weights were collected prior to vaccination, prior to challenge and at the scheduled necropsy; average daily gain (ADG) was calculated. A case definition was defined a priori to incorporate the major end point manifestations of salmonellosis: enterocolitis, lymphadenopathy and death.

Preventive fractions with 95% CI were calculated as the complement of the risk ratio (StatXact 8.0). The 95% CI for the mitigated fractions were estimated using bootstrapping methods, stratifying by pen (SAS 9.2). All hypothesis testing was conducted using an alpha level of 0.05 (SAS 9.2) and Least Square Means (LSM) are reported. Fisher’s exact test was utilized when appropriate to compare differences between treatment groups.

**Table 1: Study design table**

Group	N	Treatment Day 0 (PO')	Challenge Day 28 (IN')	Necropsy	Parameters evaluated
Placebo	24	Media	> 10 <sup>10</sup> cfu/dose	D 38	Clinical signs, average daily weight gain, diarrhea, fecal shedding, organ colonization, mortality, seroconversion, organ pathology
Vaccinate	24	ST/SC	> 10 <sup>10</sup> cfu/dose	D 38	
Strict Control	12	None	n/a	D 27	Fecal shedding, organ colonization, seroconversion, organ pathology

\* PO=per os, IN=intranasal

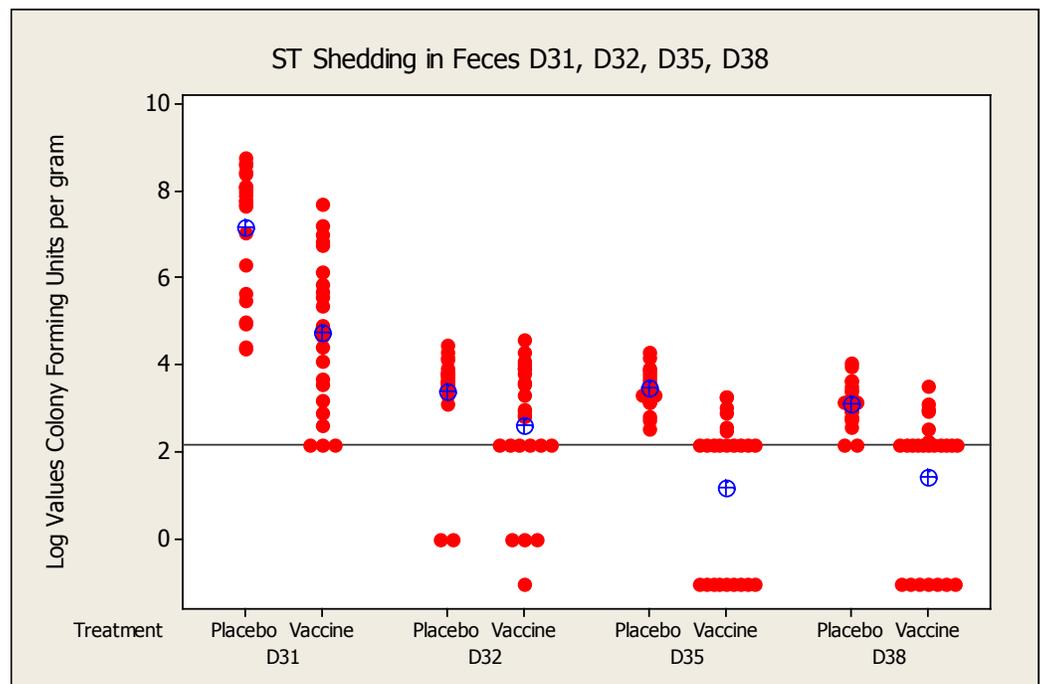
**Results**

All pigs in the strict control group were negative for any indication of colonization by *Salmonella*. Four pigs in the placebo treated group either died or were euthanized due to resultant disease post-challenge. No vaccinated pigs succumbed to severe clinical disease due to the ST challenge. Fifty percent of vaccinated pigs did not develop diarrhea at all, and 21% of the vaccinated pigs exhibited diarrhea for a single day only. Significantly more placebo treated pigs (18 of 24) exhibited diarrhea for two or more consecutive days post challenge than did vaccinated pigs (7 of 24) (Fisher’s Exact test  $p=0.0117$ ). The vaccinated group demonstrated a significant reduction in the duration of diarrhea ( $p<0.0001$ ) from 8.05 days in the placebo group to 2.54 days in the vaccinated group (Mitigated Fraction of 75% (95% CI; 47.8, 94.7)).

Following vaccination and prior to challenge, no difference in ADG between the placebo and the vaccinated group was observed (ADG=0.43 kg in both groups). However, the vaccinated pigs exhibited significantly greater ADG (0.37 kg) compared to placebo treated pigs (0.15 kg) during the post-challenge period ( $p=0.0006$ ), resulting in, on average, a 2.42 kg heavier vaccinated pig during the ten-day post-challenge period ( $p=0.0006$ ).

Blood was collected for serological testing for antibodies against *Salmonella* using the IDEXX *Salmonella* ELISA

**Figure 1:** Bacterial counts of ST shedding in feces, reported as Log cfu/gram of feces over time. The line represents the detection limit for direct quantification at 150 cfu/gram. Values at “0” represent those samples were positive only by enrichment culture. Points below the zero line represent samples that were negative by direct and enrichment culture for *Salmonella*. Mean values are denoted by circled crosses.



prior to vaccination, challenge and necropsy. One pig in each of the placebo and strict control group was positive for *Salmonella* antibodies. On the day of challenge, all placebo and strict control pigs were negative for *Salmonella* antibodies and three of the twenty-four pigs (13%) were positive in the vaccinated group. At the time of necropsy, all vaccinates had seroconverted with *Salmonella* antibodies and twelve of the twenty (60%) remaining placebo pigs had seroconverted.

When considering the parameters included in the case study definition, 15 out of 24 placebo pigs (63%) met the case definition where as only 7 of the 24 vaccinated pigs (29%) met the case definition. The reduction of disease due to ST in the vaccinated pigs was statistically significant (LSM difference  $p=0.0023$ , Preventive Fraction of 53.3% (95% CI; 7.7, 80.7)).

Post-challenge fecal samples were enumerated on D31, D32, D35 and D38 of the study. On the other days they were determined to be positive by direct or enrichment culture, or negative. As plotted in Figure 1 the vaccinated pigs had lower numbers of ST shed in the feces on each of the four days quantified. On D31 of the study, the amount of ST shed in the vaccinated pigs compared to the placebo treated pigs was reduced. As time continues the number of negative pigs in the vaccinate group increases while all of the remaining placebo pigs continue shedding through d38.

All placebo treated pigs shed the ST challenge strain for up to ten days (Table 2). Conversely, the vaccinated pigs had reduced shedding after D 31 on all but one day of the post-challenge period when compared to the placebo pigs. Vaccination reduced the duration of shedding of the ST challenge with a Mitigated Fraction of 45% (95% CI; 20.8, 72.2).

## Discussion

Post-challenge, four placebo treated pigs succumbed to challenge while all vaccinated pigs were protected from mortality. At necropsy, the placebo treated pigs had more frequent and more severe lesions suggestive of ST than the vaccinated pigs. Placebo treated pigs demonstrated significantly increased frequency and duration of diarrhea compared to the vaccinated pigs. Furthermore, there was a reduction in the amount of ST shed in the feces on three of the four days in which numbers of *Salmonella* shed was quantified. An important outcome to note is that even after a very high ST challenge, the incidence of fecal shedding by vaccinated pigs dropped to 71% within ten days while all of the placebo pigs continued to shed *Salmonella* at levels detectable by direct culture. If an infectious dose of *Salmonella* is considered to be at least 1000 cfu/gram (2), the incidence of shedding in the vaccinates after ten days drops to 8% for pigs shedding over 1000 cfu/gram, on average, while the placebo treated pigs had an incidence of 60%. Reduced shedding by vaccinates could reduce pathogen transmission, the number of subclinical salmonellosis cases, and improve pig performance. Additionally, a reduction in colonization and shedding may reduce *Salmonella* risk during lairage.

## Conclusion

Overall the vaccine significantly reduced clinical signs associated with salmonellosis including diarrhea and ST shedding. Furthermore, the post-challenge weight gain was significantly improved in vaccinated pigs. This data is supportive that a single dose of this lyophilized avirulent live vaccine containing *Salmonella* Typhimurium and *Salmonella* Choleraesuis administered in drinking water aids in the prevention of salmonellosis.

## References

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**Table 2:** Percentage of pigs with ST detected in feces by enrichment or direct culture

Group	D29	D30	D31	D32	D33
Placebo	79	100	100	100	100
Vaccinates	46	88	100	96	71
Group	D34	D35	D36	D37	D38
Placebo	100	100	100	100	100
Vaccinates	75	63	58	79	71