Reduced shedding and clinical signs of *Salmonella* Typhimurium in nursery pigs vaccinated with a *Salmonella* Choleraesuis vaccine

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Summary

Objective: To determine whether vaccination with a modified-live *Salmonella* serotype Choleraesuis vaccine can protect pigs 3 weeks of age or older against infection caused by *Salmonella* serotype Typhimurium.

Methods: Three studies were conducted using 3- to 4-week-old crossbred pigs. The studies differed in the number of times the vaccine was administered (twice in Study One, once in Studies Two and Three), the severity of the challenge (1×10^{10} in Studies One and Two, 1×10^{6} in Study Three), and the principle variables recorded during the study (clinical disease in Studies One and Two, shedding and isolation of the challenge organism in feces and tissues in Studies One and Three). In each study, pigs in a VACC-CHAL group were vaccinated orally (individually in Study One or with a water proportioner in Studies Two and Three) at 3 weeks of age and challenged 3 weeks later with virulent *Salmonella* Typhimurium. CHAL pigs were challenged but not vaccinated, and Control pigs were neither vaccinated nor challenged. Pigs were weighed and their temperatures were taken; they were scored for clinical signs of disease and for fecal consistency; and fecal, serum, and necropsy samples were taken for culture and ELISA.

Results: A significant difference in average percent healthy pigs, increased average daily gains, and lower rectal temperatures were observed in the VACC-CHAL pigs compared to CHAL pigs in Studies One and Two. Vaccinated pigs shed *Salmonella* Typhimurium for significantly fewer days than the CHAL pigs, and *Salmonella* Typhimurium was recovered from significantly more tissues and in greater numbers from nonvaccinated pigs than from the vaccinated pigs in Study Three.

Implications: A *Salmonella* Choleraesuis vaccine can aid in preventing disease and reduce the shedding and colonization of *Salmonella* Typhimurium in pigs ≥3 weeks of age.

Keywords: swine, *Salmonella*, vaccine, *Salmonella* serotype Typhimurium; *Salmonella* serotype Choleraesuis

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The two most common causes of salmonellosis in swine are *Salmonella* serotype Choleraesuis and *Salmonella* serotype Typhimurium.[1] *Salmonella* Typhimurium infections, most commonly seen in intensively reared weaned pigs, cause an enterocolitis of variable severity, followed by a carrier state that can last up to 28 weeks.[1,2] Infected pigs are believed to be the primary source of infection for other pigs and pork carcasses.

Efficacious modified-live (ML) vaccines for swine are commercially available for *Salmonella* Choleraesuis but not for *Salmonella* Typhimurium.[3] Cross protection between *Salmonella* serogroups has been demonstrated in poultry,[4] but the results of studies in other species have not been consistent. In cattle, ML vaccines administered by intramuscular (IM) injection have provided a degree of cross protection between *Salmonella* Typhimurium and *Salmonella* serotype Dublin,[5,6] and between *Salmonella* Choleraesuis and *Salmonella* Dublin.[7] However, results of studies on the vaccination of cattle with orally administered avirulent *Salmonella* Dublin have been unable to demonstrate protection against *Salmonella* Typhimurium.[8]

We are unaware of any reports of studies of cross protection between *Salmonella* Choleraesuis and *Salmonella* Typhimurium in swine. The objective of this series of three consecutive studies was to determine whether a commercially available ML Δcya Δ(crp-cdt) *Salmonella* Choleraesuis vaccine (administered either once or twice) could reduce the shedding of *Salmonella* Typhimurium and the clinical signs of disease in 3- to 4-week-old pigs after experimental challenge (at two different challenge doses) with a wild strain of *Salmonella* Typhimurium.

Materials and methods

All three studies were performed consecutively with crossbred 3- to 4-week-old pigs obtained from a commercial farm negative for fecal shedding of Salmonellae or serum antibodies to *Salmonella* Choleraesuis outer membrane proteins (OMPs). In each study, the pigs were weighed, ear tagged, and randomly allocated to one of three separate isolation rooms at the Bayer Animal Research Facility in Rushmore, Minnesota. After a 4-day acclimation period, each roomful of pigs was assigned to one of three treatment groups:

- "VACC-CHAL,"
- "CHAL," or
- "Control."

The "VACC-CHAL" group was vaccinated:

- twice (2 weeks apart) in Study One

SDC, ASA, ETT, TLS: Bayer Corporation, Agriculture Division, Shawnee Mission, Kansas 66201; GFJ: presently at Schering Plough Animal Health

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(VACC-CHAL1) individually *per os*, or
- once in Studies Two and Three (VACC-CHAL2 and VACC-CHAL3) by water proportioner

with a ML Δcya Δ(crp-cdt) *Salmonella* Choleraesuis vaccine (Argus™ SC, Bayer Corporation; Shawnee Mission, Kansas) (Table 1). To prepare vaccine, 1 mL of sterile diluent-hydrated vaccine was suspended into 9 mL of water according to the insert and label directions. Water was withheld for 6 hours prior to vaccine administration.

Approximately 3 weeks after vaccination, each pig in the VACC-CHAL group was restrained and orally given 10 mL of the adjusted challenge culture *Salmonella* Typhimurium (P93–482) using a plastic syringe, at:

- 1×10^{10} colony forming units (CFU) for Studies One and Two, and
- 1×10^{6} CFU for Study Three (Table 1).

The "CHAL" (CHAL1, CHAL2, and CHAL3) group received the *Salmonella* Typhimurium challenge on the same day of the study as the VACC-CHAL pigs received their challenge, but no vaccine.

The "Control" (Control1, Control2, Control3) group received neither vaccine nor challenge.

To prepare the challenge dose, a frozen vial of the challenge strain was thawed in a 98.6°F (37°C) water bath. One mL of the thawed culture was transferred to a 2-L Erlenmeyer flask which contained 500 mL of MLB (Modified Luria Bertani). After overnight incubation of static growth at 37°C ± 2°C (98.6°F ± 3.6°F), aliquots of this culture were adjusted with sterile saline solution to the desired concentration, and stored in an icewater bath until administered to the pigs.

Feed was withheld from the pigs for 18 hours prior to challenge and returned to the pigs 30 minutes after challenge-exposure in each of the studies.

Sampling and data collection

During the prevaccination acclimation period, normal "baseline" scores for physical condition and fecal consistency were made for each pig. Fecal consistency and physical condition were evaluated daily after challenge for each pig on a scale of 1 (normal) to 4 (most severe). In addition, fecal and serum samples were collected and evaluated by culture and ELISA to determine whether the pigs were free of *Salmonella*.

Pigs were individually weighed three times: prior to vaccination, prior to challenge, and prior to necropsy, and the average daily gain (ADG) for the postchallenge period was calculated.

Physical condition scores and fecal consistency scores were recorded daily starting 2 days before challenge through day 10 postchallenge, and on days 12–14 postchallenge for each animal.

The rectal temperature of each pig was recorded daily after challenge, and the maximum and mean rectal temperatures were determined. The maximum rise in temperature for each pig was calculated as the difference between the maximum postchallenge and the mean prechallenge normal rectal temperatures.

Fecal samples were collected daily (in Studies One and Three only) for culture (Table 1). In addition, approximately 1 g each of liver, spleen, ileo-cecal junction (ICJ), tonsils, and mesenteric lymph node (MLN) were collected at necropsy.

Blood samples were collected postvaccination and postchallenge. Sera were stored at -20°C until tested for antibodies to *Salmonella* Choleraesuis (prevaccination and postvaccination) and *Salmonella* Typhimurium (postchallenge) OMPs using an enzyme-linked-immunosorbent-assay (ELISA).

Mortality was recorded throughout each study.

Culture

Aliquots of fecal samples and homogenized tissues were plated onto brilliant green agar plates (BGA), both directly (after serial dilution with phosphate-buffered saline) and after enrichment in Rappaport-Vassiliadis Broth (Difco; Detroit, Michigan) at 37°C ± 0.55°C (98.6°F ± 1°F) for 18–24 hours. Plates were incubated at 37°C ± 0.55°C (98.6°F ± 1°F) for 24 and 48 hours and examined for pink colonies typical of *Salmonella* spp. Presumptive identification was

Table 1: Experimental design

	Study One	Study Two	Study Three
Pigs/group			
VACC-CHAL	10	20	21
CHAL	10	20	19
Control	5	5	5
Vaccination			
Route	Oral - individually *per os*	Oral - mass via water proportioner	Oral - mass via water proportioner
Frequency	Twice	Once	Once
Challenge			
Dose/pig	10^{10} CFU	10^{10} CFU	10^{6} CFU
Samples/measures taken			
Mortality	x	x	x
Physical condition scores	x	x	x
Fecal scores	x	x	x
Weight	x	x	x
Temperature	x	x	x
Fecal samples	x		x
Necropsy samples	x		x

based on standard biochemical tests and slide agglutination with *Salmonella* 'O' Group B antisera (Difco).

Serology

Sarkosyl-insoluble OMPs were isolated and dissolved in phosphate-buffered saline (PBS, pH 7.2), diluted in sodium bicarbonate buffer, and used to coat plate wells. The coated wells were incubated overnight at 4°C, washed with PBS-Tween 20 (0.05%), and blocked with PBS-bovine serum albumin (BSA, 0.05%). Aliquots (100 µL) of serially diluted (1:100–1:3200) serum samples were added to the OMP-coated wells, in duplicate. After 1 hour of incubation at room temperature, the wells were washed four times with PBS-Tween 20. Aliquots (100 µL) of a solution of horseradish peroxidase-labeled anti-swine IgG in PBS-BSA were added to the wells. The plates were incubated for 1 hour at room temperature after which the wells were washed four times with PBS-Tween 20. Aliquots (100 µL) of a solution of ABTS-hydrogen sulfide (substrate) were added to the wells and the plates were incubated at room temperature for 30 minutes. An aliquot (50 µL) of 2% sodium dodecyl sulphate (SDS) solution was added to each well after which the optical density (OD) of each well was read at 405 nm wavelength. The OD of the blank wells was subtracted from the OD from each of the test wells.

Statistical analysis

The number of days a pig was recorded with a fecal score of 1 were proportionalized by dividing with the total number of observation days postchallenge. This proportion is referred to as the percent "normal" days per pig. Similarly for the physical condition scores, a percent "healthy" days per pig variable was determined from pigs that received a score of 1 and proportionalized by dividing with the total number of postchallenge observation days.

Also, the number of days a pig was shown shedding *Salmonella* was proportionalized by dividing with the total number of days the pig was culture-tested postchallenge. This proportion is referred to as the percent shedding days. Second, the number of pigs per day that *Salmonella* was isolated from the feces was also determined and proportionalized by dividing with the total number of pigs per group per day. This proportion is referred to as the percent animals shedding per day.

Variables derived from fecal scores; physical scores; fecal shedding; average daily gains; maximum, mean, and maximum rise in body temperatures; and percent tissues positive were analyzed using ANOVA, testing for group effects, provided tests for normality and homogeneity of variances were met. Wilcoxon's rank sum test was applied where criteria for ANOVA were not met. Similarly, rectal temperatures recorded postchallenge were also analyzed using repeated-measures ANOVA.

Results

The Control pigs (Control1, Control2, and Control3) remained clinically normal in all studies. *Salmonella* Typhimurium was isolated once from the tonsils and feces of one Control1 and one Control3 pig. However, no Salmonellae were isolated from the feces or tissues of Control2 pigs.

There was no significant increase in the postvaccination antibody titers of the VACC-CHAL pigs in any of the three studies. CHAL1 and CHAL2 pigs developed clinical signs of salmonellosis (inappettance, lethargy, and/or gauntness with or without rough hair coat), and in all studies *Salmonella* Typhimurium was detected in the feces of all CHAL pigs, demonstrating that *Salmonella* Typhimurium infection was established in the pigs that were challenged.

Study One

The VACC-CHAL1 and CHAL1 pigs had group mean body temperatures above 40°C (104°F) only during the first 3 days after challenge. The mean body temperature of the VACC-CHAL1 pigs on days 2 and 3 postchallenge were significantly ($P < .05$) lower than those of the CHAL1 pigs, (Figure 1).

VACC-CHAL1 pigs had a significantly ($P < .05$) greater percent of healthy pigs per day after challenge than did the CHAL1 pigs (Figure 2). Also, the percent of VACC-CHAL1 pigs with normal fecal scores was significantly ($P < .05$) greater than the CHAL1 pigs (Figure 3). The ADG of the VACC-CHAL1 pigs was significantly ($P < .05$) greater than that of the CHAL1 pigs (Figure 4).

Figure 1: Mean rectal temperature per treatment

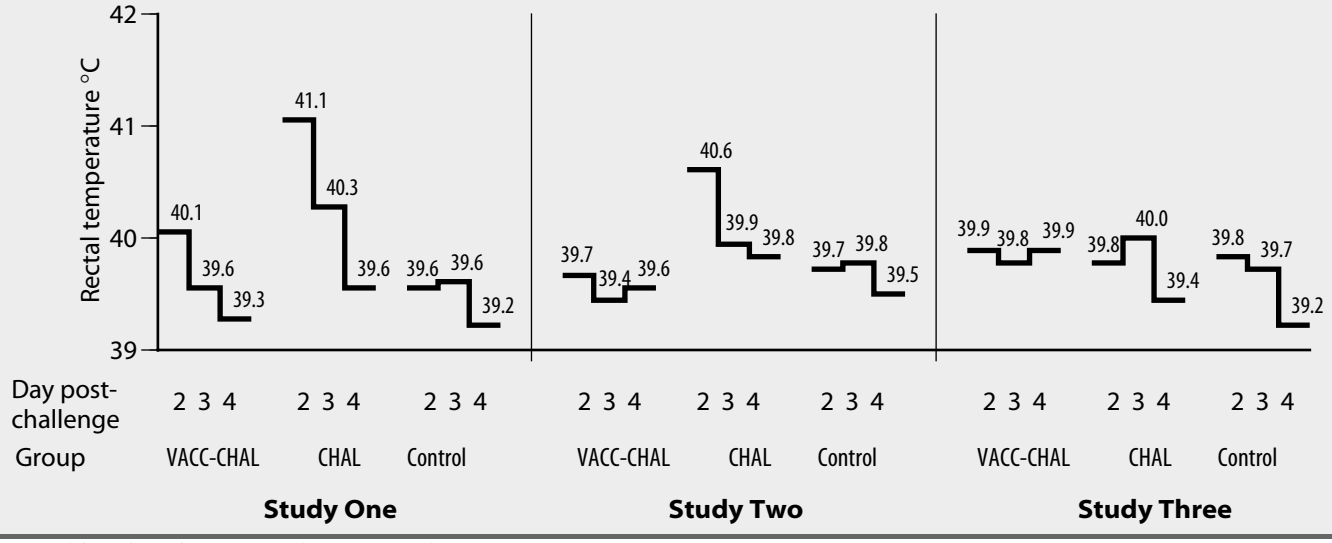


Figure 2: Mean percent healthy pigs per day

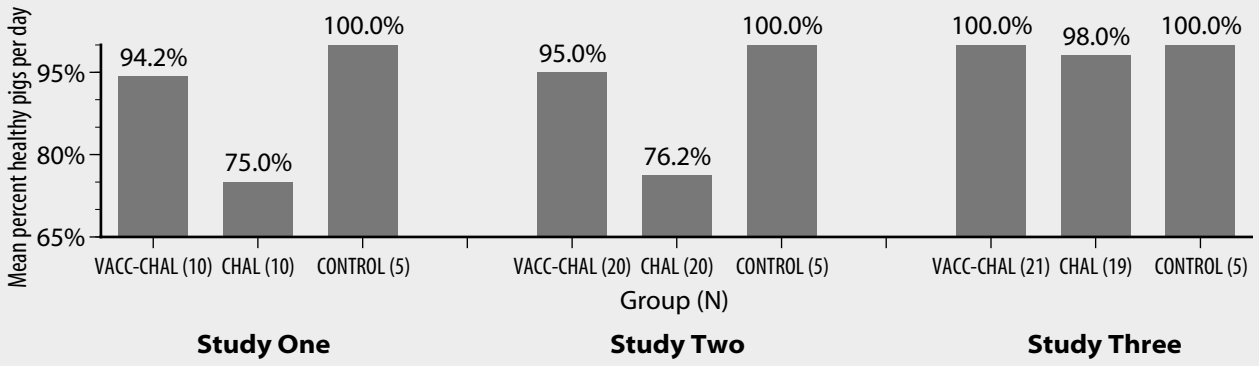


Figure 3: Mean percent pigs with normal fecal score

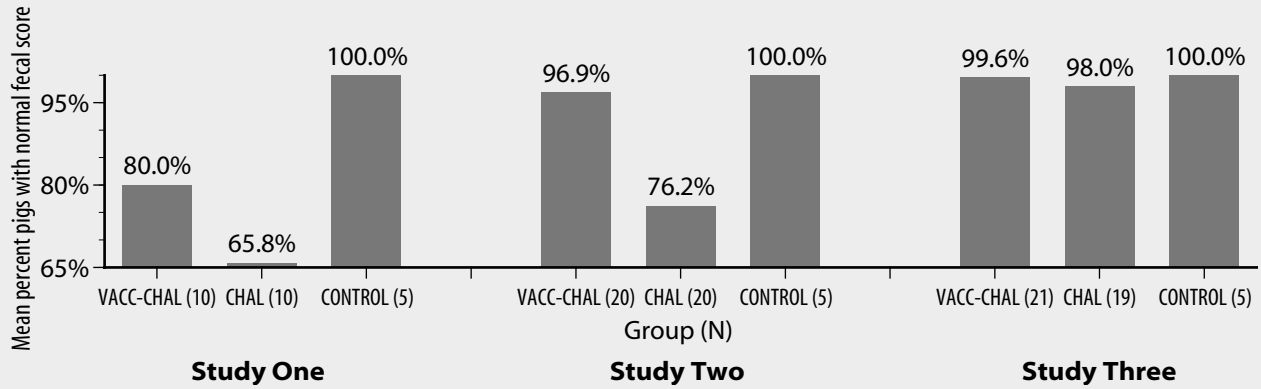
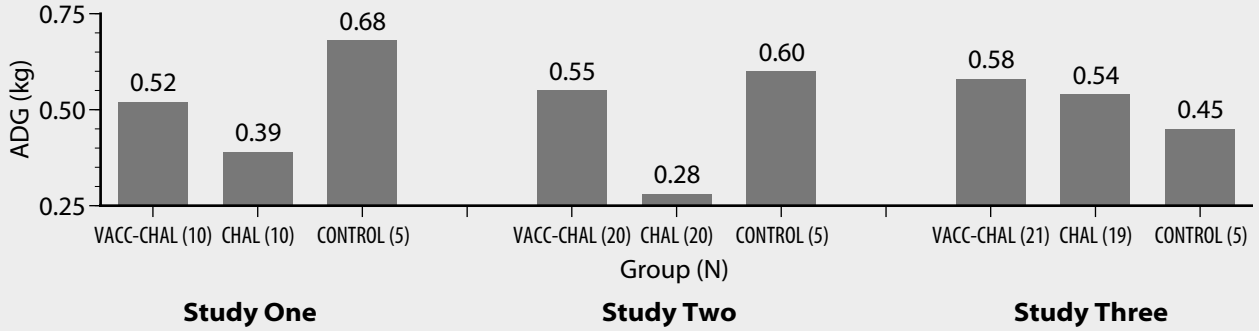


Figure 4: Average daily gain



Although there was no statistical difference between the two groups in the number of pigs that shed *Salmonella* Typhimurium or had *Salmonella* Typhimurium in their tissues, VACC-CHAL1 pigs had fewer *Salmonella* Typhimurium in their tissues than the CHAL1 pigs (Figure 5).

Study Two

Four pigs died after challenge, three in the CHAL2 group and one in the VACC-CHAL2 group.

Body temperatures in both the VACC-CHAL2 and CHAL2 pigs were elevated above normal values 24 hours after challenge with virulent *Salmonella* Typhimurium (Figure 1). The mean body temperature of the VACC-CHAL2 pigs returned to normal within 48 hours after challenge, but that of the CHAL pigs remained above normal. On days 2, 3, and 4 postchallenge, the mean body temperatures of the VACC-CHAL2 pigs were significantly lower ($P < .05$) than those of the CHAL2 pigs. Mean rectal temperatures, maximum rectal temperatures, and the rise in rectal temperatures did not differ significantly between the VACC-CHAL2 and the nonvaccinated groups postchallenge.

The average percent of healthy pigs was significantly greater ($P < .05$) in the VACC-CHAL2 group than in the CHAL2 group (Figure 2). The average percent of vaccinated pigs with normal fecal scores was significantly ($P < .05$) greater than in the CHAL2 group (Figure 3). The average daily gain of the VACC-CHAL2 pigs was significantly ($P < .05$) greater than that of the CHAL2 pigs (Figure 4).

Study Three

Pigs developed only mild and transient clinical signs of disease in Study Three (Figure 2). As a result, group differences in average daily gain, body temperature, and clinical signs were not significant in Study

Three.

Significantly more CHAL3 pigs (15 of 19) than VACC-CHAL3 pigs (two of 21) were culture-positive for *Salmonella* Typhimurium in at least one tissue ($P < .01$) at necropsy (Figure 5). *Salmonella* Typhimurium was isolated from a significantly greater proportion of tissues from the CHAL3 pigs than from the VACC-CHAL3 pigs (10.0%, $P < .05$). A higher percent of CHAL3 pigs were positive with the challenge organism in tonsils, ICJ and MLN when compared with the VACC-CHAL3 pigs.

The CHAL3 pigs were detected shedding *Salmonella* Typhimurium significantly ($P < .05$) more days than the vaccinated pigs postchallenge. A significant ($P < .01$) number of CHAL3 pigs (11) were detected shedding *Salmonella* Typhimurium in feces than the vaccinates (zero) on the last day of the observation period (Figure 6). None of the vaccinated pigs shed *Salmonella* Typhimurium in feces beyond day 9 postchallenge, while 11 of the 19 CHAL3 pigs were still shedding *Salmonella* Typhimurium in feces on day 14. The mean number of *Salmonella* Typhimurium in the feces of the CHAL3 pigs (7.5 per g) was significantly greater ($P < .05$) than the number in the VACC-CHAL3 pigs (0 per g) on day 7 postchallenge, and was higher on day 9 postchallenge (2.5 per g versus 0 per g) (Figure 7).

Discussion

The clinical signs, body temperature, and culture results in the respective vaccinated pigs of studies One and Two were similar to other reports of experimental *Salmonella* Typhimurium infection of weaned pigs challenged with approximately the same dose of virulent bacteria.[2,10] In those studies, as in these, the mean rectal temperatures peaked in the first 2 days postchallenge and were elevated until day 4 or later, diarrhea was seen in 80%–90% of the nonvaccinated challenged pigs, and clinical signs varied from mild to severe.

Isolation of *Salmonella* Typhimurium in Study Three was primarily from the tonsils, ICJ, and MLN of the nonvaccinated pigs. This is consistent with the previous studies in which deep tissues (liver and spleen) were rarely infected, but mucosal and lymphoid tissues were commonly infected.[2,10] The serological data (data not shown)

Figure 5: Percent positive for *Salmonella* Typhimurium at necropsy by tissue type

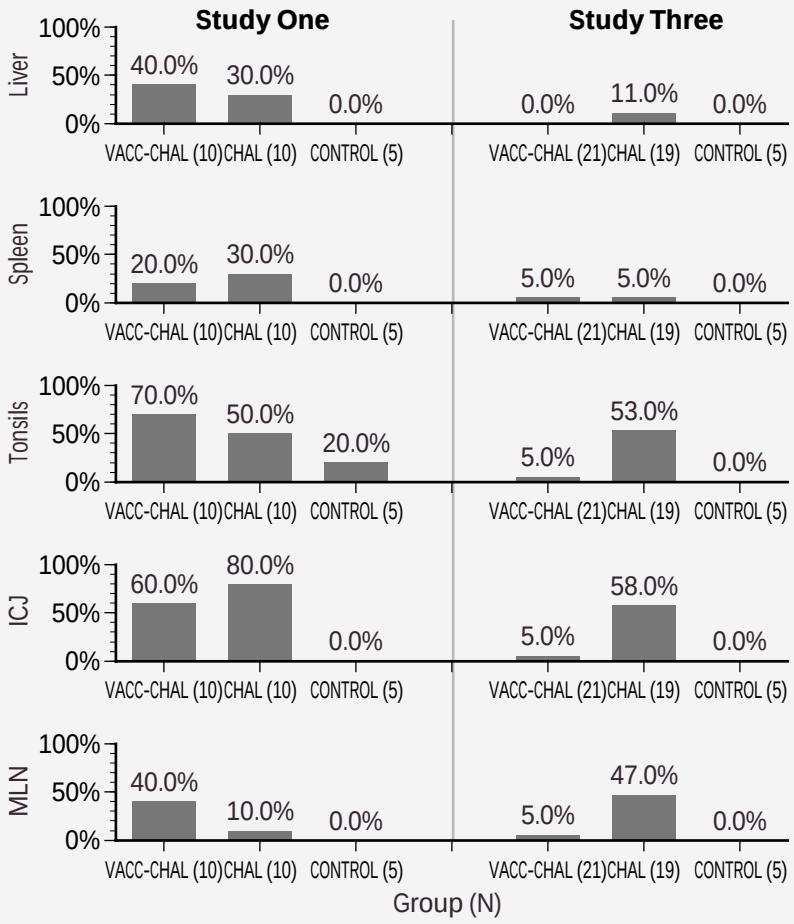
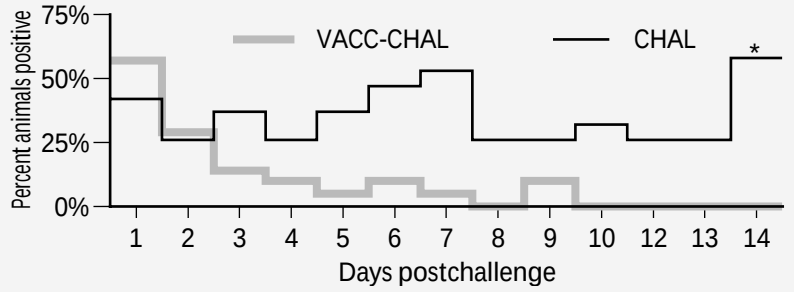
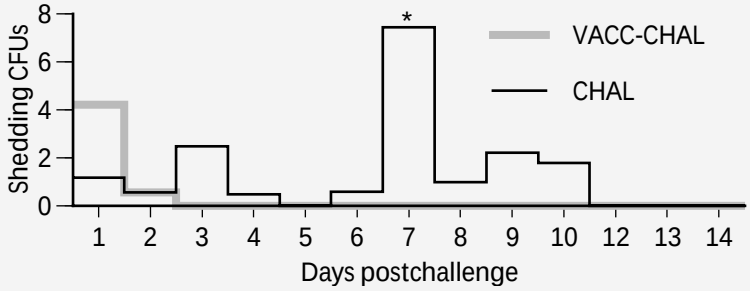


Figure 6: *Salmonella* Typhimurium shedding in Study Three



* $P < .01$ on day 14

Figure 7: Geometric mean of *Salmonella* Typhimurium shedding in Study Three



* $P < .05$ on day 7

indicated that the immunoglobulin- (IgG-) mediated response to the *Salmonella* Choleraesuis OMP antigens, induced by the vaccine delivered orally, was minimal and did not differ significantly from that of the unvaccinated animals. This may explain the lack of humoral response to either vaccination or infection in the vaccinated and nonvaccinated pigs, as the immunity at the mucosal level is largely mediated by secretory immunoglobulin A and cell-mediated immunity.[11]

The differences between the clinical signs of disease seen in vaccinates and nonvaccinates in studies One and Two were similar to those described in a report of an experimental Δcya Δ(crp-cdt) *Salmonella* Typhimurium vaccine.[10] The gene deletions in that vaccine are also among the deletions [Δcya Δ(crp-cdt)] present in the *Salmonella* Choleraesuis vaccine used in these studies. In that report, as in these studies, the mean body temperatures of vaccinated pigs returned to normal by 48 hours after challenge, and significantly fewer (50%) of the vaccinated pigs than nonvaccinates (90%) developed diarrhea. The vaccinated pigs in that study were lethargic and demonstrated a depressed appetite, but returned to normal by 48 hours after challenge, while similar measures of the nonvaccinated pigs were more severe and required longer to return to normal.

Studies in cattle with oral vaccination with Δaro A Δaro D *Salmonella* Dublin vaccines did not demonstrate cross protection between *Salmonella* Typhimurium and *Salmonella* Dublin.[8,9] The attenuating mutations in those vaccines severely limit the growth of the bacteria in mammalian tissues, and *Salmonella* Choleraesuis is genetically more similar to both *Salmonella* Typhimurium and *Salmonella* Dublin than either is to each other.[12] The host species difference, the difference in the attenuating mutation, or the difference in serovars used may all be responsible for the disparate results.

Although *Salmonella* spp. may survive for long periods in the environment, it is widely believed that the carrier animal is the major source of infections for both animals and humans.[1] The significant reduction in shedding of *Salmonella* Typhimurium, and the lower prevalence of the challenged organism in the tissues suggests that vaccination with the Δcya Δ(crp-cdt) *Salmonella* Choleraesuis vaccine would be a useful tool in preventing salmonellosis in swine.

Implications

- A commercially available Δcya Δ(crp-cdt) *Salmonella* Choleraesuis modified-live vaccine administered as per the label directions can aid in reducing shedding and clinical signs of *Salmonella* Typhimurium in pigs ≥ 3 weeks of age.
- A significant degree of cross protection between the *Salmonella* Choleraesuis vaccine and *Salmonella* Typhimurium has been demonstrated.

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