Passive Immunity Stimulated by Vaccination of Dry Cows with a *Salmonella* Bacterial Extract

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Background: Diarrhea because of *Salmonella* infection is a cause of neonatal calf diarrhea. The stimulation of passive immunity in the calf by vaccinating the dam for *Salmonella* has shown some success in previous studies; however, there are no data on the use of currently licensed vaccines in the United States.

Objective: To determine whether vaccinating cows at dry-off with a commercially available *Salmonella* bacterial extract would stimulate *Salmonella*-specific antibodies in the colostrum of cows at calving and whether these antibodies would be transferred to the calf.

Animals: Sixty Holstein cattle and 59 calves from a herd presumed to be naïve to *Salmonella*.

Methods: Prospective clinical trial. Thirty cows were vaccinated at dry-off with a *Salmonella enterica* serovar Newport bacterial extract and again 4 weeks later. An additional 30 cows received only saline. Calves fed fresh colostrum from their dam within 4 hours of birth had blood collected 24 hours later.

Results: Vaccinated cattle had increased *Salmonella* Newport antibody titers at calving in blood ($P = .01$) and colostrum ($P = .011$). Calves that received colostrum from vaccinated cattle also had significant increase in *Salmonella* antibodies (1.04 ± 0.03) as compared to calves born to unvaccinated cows (0.30 ± 0.02).

Conclusions and Clinical Importance: The results indicate that the use of a commercially available *Salmonella* vaccine can stimulate antibodies that are passed on to the calf via colostral transfer. Further studies need to be done to determine whether these antibodies will offer protection against *Salmonella* challenge.

Key words: Calves; Colostrum; IgG; Salmonella Newport; SRP.

*S*almonella infections are an economically important disease of cattle and also represent a worldwide public health concern. Although cattle of all ages can be infected with *Salmonella* bacteria, death is most often reported in calves less than 8 weeks of age.[1,2] The science behind the vaccination of cattle for different serotypes of *Salmonella* has progressed in the past 20 years[1] and 2 commercially available vaccines are currently on the market in the United States for use in cattle. However, neither of these vaccines is approved for calves less than 2 weeks of age and diarrhea because of *Salmonella* in neonatal calves still remains a problem. This has led some veterinarians to use vaccines in young calves by unapproved routes of administration, which has largely proven ineffective.[3] Another potential approach to achieving at least partial immunity in young calves is through vaccination of the dam. This has long been a common practice for other causes of neonatal calf diarrhea including *E. coli*, rotavirus, and coronavirus with established efficacy.[4,5] Previous studies suggested that the use of passive immunity through vaccination of the dam or via the use of *Salmonella*-specific antibodies derived from egg

yolks could offer at least partial protection against clinical disease in calves.[6–8] Therefore, the purpose of this study was to determine whether vaccinating cows at dry-off with a commercially available *Salmonella* bacterial extract would result in the presence of *Salmonella*-specific IgG antibodies in the colostrum of cows at calving and whether these colostral antibodies would be transferred to the calf.

Materials and Methods

Animals and Experimental Design

This study was approved by the Institutional Animal Care and Use Committee at North Carolina State University. Sixty lactating Holstein cattle ranging from 3 to 8 years of age were identified for this study according to computer records (Table 1). All cattle were housed on a single dairy farm in North Carolina. This farm had never used an autogenous or commercially available *Salmonella* vaccine, and there had not been a diagnosis of clinical salmonellosis on the farm for at least 10 years. Although extensive testing had not been done to rule out the presence of subclinical salmonellosis in the herd, the farm had extensive diagnostic testing records of both calves and adult cattle. This data included fecal cultures from healthy animals as well as those with diarrhea along with numerous necropsy reports spanning a period of 10 years. During this time period, there had never been a positive *Salmonella* culture on the farm. A search of the farm's records identified the next 60 cows scheduled for dry-off. These cows were randomly allocated to serve in either "vaccinated" or "control" groups by assigning every other cow in the computer-generated list to opposite groups. Thirty cows were allocated to a group that was vaccinated at the end of lactation (dry-off). During the week, the cow was scheduled to be dried off (8-week dry period), 10 mL of blood was collected and each cow was vaccinated with 2 mL of a commercially available *Salmonella enterica* serotype Newport bacterial extract[a] given SC in the neck. The vaccine has a conditional license in the United States and is approved for use in cattle 6 months of age and older. An additional 30 cows were allocated to the control group and received

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Table 1. Summary of age and production history for cows included in this study. The data listed were collected from farm records on the 1st day of the experimental period (dry-off).

	Age of Cow (years)	Lactation Number	Days in Milk (Current Lactation)	305-day Mature Equivalent (kg)
Control group	5.2 ± 2.8	2.5 ± 1.7	362 ± 24	10,450 ± 1,290
Vaccinated group	5.6 ± 2.3	2.9 ± 1.5	371 ± 35	10,280 ± 1,710

2 mL of saline SC in the neck during the week of dry-off. Cows were identified with neck bands by group at the time of initial vaccination. Four weeks after the initial injection, all cows in the "vaccinated" group received a 2nd dose of (2 mL) *Salmonella* Newport vaccine, whereas the control group again received saline. Cows were housed together in a grass calving lot during the dry period, and were fed a total mixed ration (TMR).

At the time of calving, colostrum was harvested within 4 hours of parturition and a 2nd 10-mL blood sample was collected from all cows. A 50-mL sample of colostrum was saved from each cow, and then all calves were fed at least 3 L of colostrum from their dam within 4 hours of birth (calves were fed 4 L unless the cow did not produce enough colostrum). Blood samples were then collected from each calf 24–48 hours after colostrum administration.

Salmonella *Serology*

Serum and colostrum antibody titers for *Salmonella* Newport were determined by an enzyme-linked immunosorbant assay (ELISA). Personnel performing the ELISA assays were not aware of group assignment for any of the animals. *Salmonella* Newport SRP antigen was coated on ELISA plates[b] at 250 ng/well in carbonate coating buffer (pH 9.6), covered and incubated overnight at 4°C. Plates were emptied and patted dry, then blocked using 200 μL/well of 1% PVA/PBS, covered and incubated 2 hours at 37°C. Dilutions of the serum and colostrum samples were prepared in 1% PVA/PBS at 1 : 1,000. Samples were tested in duplicate. Plates were covered and incubated at 37°C for 1 hour. Plates were washed 3 times with 0.05% PBS-Tween 20. After this wash step, 100 μL of conjugate sheep anti-bovine IgG[c] in 1% PVA/PBS was added to each well. Plates were covered and incubated at 37°C for 1 hour. Plates were washed as before and developed using 100 μL of 2,2' azino-di-3-ethyl-benzthiazoline-6-sulfonate (ABTS),[d] and the absorbance was read at 405/490 nm with an ELISA reader.[e] Results were reported as the sample to positive (S : P) ratios. The average of the negative controls' optical density was calculated and subtracted from all values as a reagent blank. Individual test variability was factored out by analyzing the optical density (OD) reading and factored out a known positive and negative OD reading from the same test. Then the sample duplicates were averaged and divided by the positive control average yielding the S : P titer.

Statistical Analysis

Data are presented as mean ± SD. Serologic titers for vaccinated and unvaccinated groups, as well as calves born to cows from each group, were compared using one-way ANOVA. Values of $P < .05$ were considered significant. A statistical software package was utilized to conduct the data analysis.[f]

Results

All 60 cows calved successfully and had colostrum samples collected. There were no differences in *Salmonella* titers between groups of cows at the end of lactation. Cows in the vaccinated group had a significant increase in antibody titers at the time of calving as compared to unvaccinated cows (Table 2). The colostrum collected from cows that had been vaccinated for *Salmonella* Newport also had significantly higher antibody titers than did colostrum from unvaccinated cows.

Twenty-nine calves born to control cows received colostrum in this study (1 was dead at birth), and 30 calves born to vaccinated cows received colostrum. Two calves were dead at birth, but 2 cows had twins and both calves were fed colostrum from the dam. Stillbirth rates were therefore 2/30 (6.7%) in the vaccinated group and 1/30 (3.3%) in the unvaccinated group. Calves that received colostrum from vaccinated cows had significantly higher *Salmonella* Newport titers as compared to calves born to unvaccinated cows (Table 2).

Discussion

This study indicates that the vaccination of late gestation cattle with a commercially available *Salmonella* Newport vaccine can result in the presence of these antibodies in colostrum and absorption by the newborn calf. Whether these antibodies would offer significant protection against *Salmonella* infections in the

Table 2. *Salmonella* Newport (SRP) antibody titers in both cows and colostrum from cows that were vaccinated twice with *Salmonella* Newport bacterial extract ("vaccinated group") in late gestation as compared to cows that were not vaccinated ("control group"). Also included are *Salmonella* titers from calves born to these cows 24 hours after drinking maternal colostrum. Data are presented as sample to positive (S/P) ratio percentage.

	Before Vaccination (at Dry-Off)	After Vaccination (at Calving)	Colostrum	Calves
Control group	0.26 ± 0.03	0.16 ± 0.02	0.66 ± 0.03	0.30 ± 0.02
Vaccinated group	0.20 ± 0.02	0.69 ± 0.03	1.49 ± 0.02	1.04 ± 0.03
P value	.56	.01	.011	.003

first few weeks of life remains to be determined. Although limited research has been done to this point looking at the ability of passive immunization to offer protection against *Salmonella* infections early in life, this suggests that this approach may offer some potential. In a study done in the 1960s, calves that received colostrum from cows vaccinated with a killed *S.* Typhimurium vaccine showed a reduction in fecal shedding of *Salmonella* after challenge.[6] In another study, a group of dairy cows were vaccinated 2 and 7 weeks before calving with a killed *S.* Typhimurium vaccine, whereas another group was left unvaccinated.[7] Calves were divided into several groups: those allowed to nurse colostrum from a vaccinated dam for 48 hours and then fed colostrum from the same dam throughout the challenge period (VC/VM); calves allowed to nurse colostrum from a vaccinated dam for 48 hours and then fed colostrum from an unvaccinated cow throughout the study (VC/NM); cows that suckled an unvaccinated dam for 48 hours and then were fed colostrum from a vaccinated cow after day 2 (NC/VM); and calves that suckled an unvaccinated cow and were fed colostrum from unvaccinated cows through the challenge period (NC/NM). All calves were challenged with *S.* Typhimurium on day 5. Death rates were significantly reduced in cows that were allowed to suckle cows that had been vaccinated before calving (0% and 22% in the VC/VM and VC/NM groups) as compared to calves that received colostrum from unvaccinated cows (75% and 50% death rate in the NC/NM and NC/VM groups). Fecal shedding of *Salmonella* organisms was also shorter in calves that nursed colostrum from vaccinated cows.

In a study done in Japan on Holstein calves from *Salmonella*-free farms, feeding *S.* Typhimurium or *S.* Dublin antibodies derived from chicken egg yolks were able to offer dose-dependent protection against an experimental challenge at 4 days of age.[8] All calves that were challenged with *Salmonella* but did not receive the egg yolk antibodies died. In calves that received a "low" dose of antibodies, death rate was reduced from the control group, but still well over 50%; however, calves that received the higher titer of antibodies had fever and diarrhea, but no deaths.

The majority of research has indicated that protection from *Salmonella* vaccination is limited to a single strain or other closely related strains of *Salmonella*. However, more recent advancements have led to vaccines that offer cross-protection against multiple bacterial strains across different *Salmonella* serogroups. Some of these are modified live vaccines that contain mutations in different regions of the bacterial genome.[9] The vaccine used in this trial is a subunit vaccine that is composed of purified extracts of siderophore receptors and porins (SRP).[10] These SRP proteins are shared by all *Salmonella* organisms and are critical for iron acquisition by the bacteria. The goal of the vaccine is to inactivate SRP proteins by antibody binding to restrict the ability of the bacteria to gain iron from the environment. Therefore, the vaccine has the potential to induce immunity to multiple serogroups of

Salmonella. Further research is necessary to determine whether or not this vaccine would be able to prevent *Salmonella* infections in calves and what strains it would be effective against.

Similar work has been done in calves with an *E. coli* SRP vaccine. Groups of beef cows were given either an E. coli O157 : H7 SRP vaccine or saline placebo approximately 60 and 30 days prior the start of calving season.[11] Calves born to vaccinated cows had increased titers of anti-*E. coli* O157 : H7 antibodies at branding time; however none of the calf groups had any difference in feedlot health, performance, or prevalence of O157 : H7 shedding upon entry to the feedlot or at slaughter.

Modified live *Salmonella* vaccines induce a broad immune response via stimulation of cell-mediated, humoral, and mucosal immunity similar to the response after natural infection.[1] For example, an aromatic amino acid (aro) auxotroph *Salmonella Dublin* vaccine is commercially available in the United States[g] and can be given to calves beginning at 2 weeks of age. Experimental studies with aro minus vaccines have demonstrated good protection against several different *Salmonella* serovars when calves were challenged within 3 weeks after vaccination.[12] The current dilemma is providing some immunity to young calves when *Salmonella* challenge occurs within the first few weeks of life. The use of dry cow vaccination to stimulate colostral antibodies that can be absorbed by the calf at birth offers a way to provide some degree of protection to these calves until they are old enough to be given a modified live vaccine. This study suggests such a strategy is possible; however, further challenge studies are needed to confirm that the presence of *Salmonella* antibody in the calves will actually provide a significant benefit from bacterial challenge.

in this study. His role was to run the serologic ELISA assays on randomly numbered tubes of serum. Geof Smith is an Associate Editor with the Journal of Veterinary Internal Medicine.

References

1. Mohler VL, Izzo MM, House JK. *Salmonella* in calves. Vet Clin Food Anim 2009;25:37–54.

2. Smith BP, Habasha FG, Reina-Guerra M, et al. Immunization of calves against salmonellosis. Am J Vet Res 1980;41:1947–1951.

3. Habing GG, Neuder LM, Raphael W, et al. Efficacy of oral administration of a modified-live *Salmonella Dublin* vaccine in calves. J Am Assoc Vet Med 2011;238:1184–1190.

4. Snodgrass DR, Nagy LK, Sherwood D, et al. Passive immunity in calf diarrhea: Vaccination with K99 antigen of enterotoxigenic *Escherichia coli* and rotavirus. Infect Immun 1982;37:586–591.

5. Crouch CF, Oliver S, Hearle DC, et al. Lactogenic immunity following vaccination of cattle with bovine coronavirus. Vaccine 2001;19:189–196.

6. Royal WA, Robinson RA, Duganzich DM. Colostral immunity against salmonella infection in calves. New Zeal Vet J 1968;16:141–145.

7. Jones PW, Collins P, Aitken MM. Passive protection of calves against experimental infection with *Salmonella typhimurium*. Vet Rec 1988;123:536–541.

8. Yokoyama H, Peralta RC, Umeda K, et al. Prevention of fatal salmonellosis in neonatal calves using orally administered chicken egg yolk *Salmonella*-specific-antibodies. Am J Vet Res 1998;59:416–420.

9. Mohler VL, Heithoff DM, Mahan MJ, et al. Cross-protective immunity conferred by a DNA adenine methylase deficient *Salmonella enterica* serovar Typhimurium vaccine in calves challenged with *Salmonella* serovar Newport. Vaccine 2008;26:1751–1758.

10. Hermesch DR, Thomson DU, Loneragan GH, et al. Effects of a commercially available vaccine against *Salmonella enterica* serotype Newport on milk production, somatic cell count, and shedding of *Salmonella* organisms in female dairy cattle with no clinical signs of salmonellosis. Am J Vet Res 2008;69:1229–1234.

11. Wileman BW, Thomson DU, Olson KC, et al. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. J Food Prot 2011;74:1599–1604.

12. Smith BP, Reina-Guerra M, Hoiseth SK, et al. Aromatic-dependent *Salmonella typhimurium* as modified live vaccines for calves. Am J Vet Res 1984;45:59–66.