**Short Communication: Characterization of the serologic response induced by vaccination of late-gestation cows with a *Salmonella* Dublin vaccine**

Geof W. Smith,*† Feli Smith,‡ Sjoert Zuidhof,∥ and Derek M. Foster*  
*Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh 27607  
†Division of Laboratory Animal Resources, Duke University, Durham, NC 27708  
‡Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO 64506

**ABSTRACT**

Diarrhea due to *Salmonella* infection is an important cause of neonatal calf diarrhea. The acquisition of passive immunity in the calf by vaccinating the dam has shown some success in previous studies; however, no data exists on the use of currently licensed vaccines in the United States. Therefore, the purpose of this study was to determine whether vaccinating cows in late gestation with a commercially available *Salmonella* Dublin vaccine would stimulate *Salmonella*-specific antibodies in the colostrum of cows at calving and whether these antibodies would be transferred to the calf. Thirty Holstein cows were vaccinated 3 wk before the end of lactation with a *Salmonella enterica* serovar Dublin vaccine, with a second dose given at dry-off. An additional 30 cows received only saline. Calves had a blood sample collected immediately after birth and were then fed fresh colostrum from their dam within 2 h of calving. A postcolostrum blood sample was collected 24 to 48 h later. *Salmonella* Dublin antibodies in colostrum as well as serum from the cows and calves were measured using an ELISA technique. Results of this study showed that vaccinated cattle had elevated *Salmonella* Dublin antibody titers at the time of calving (40.3 ± 9.1) as compared with control cows (−9.4 ± 1.1). Calves that received colostrum from vaccinated cattle also had a significant increase in *Salmonella* Dublin antibodies (88.5 ± 8.9) as compared with calves born to unvaccinated cows (−3.2 ± 1.2). This study demonstrated that the use of a commercially available *Salmonella* Dublin 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:** calf, colostrum, immunoglobulin G, *Salmonella* Dublin, passive immunity
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 yr. 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 yr. During this period there had never been a positive *Salmonella* culture on the farm. A search of the farm 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 3 wk before the scheduled dry-off date. A total of 10 mL of blood was collected, and each cow was vaccinated with 2 mL of a commercially available modified live *Salmonella enterica* serotype Dublin bacterin (Entervene-d, Boehringer Ingelheim Vetmedica, St. Joseph, MO) given subcutaneously in the neck. The vaccine is approved in the United States for cattle greater than 2 wk of age. An additional 30 cows were allocated to the control group and received 2 mL of saline subcutaneously in the neck. Cows were identified with neck bands by group at the time of initial vaccination. Rectal temperature was monitored before vaccination as well as 2, 4, 12, and 24 h after vaccination. In addition, daily milk production from both groups was recorded for 2 wk after the initial vaccination, and any abortions or stillbirths throughout the study period were noted. Three weeks after the initial injection (on the actual dry-off day), all cows in the vaccinated group received a second injection dose of (2 mL) vaccine, and the control group again received saline. Cows were housed together in a grass calving lot during the 8-wk dry period.

At the time of calving, colostrum was harvested within 2 h of parturition, and a 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 4 L of colostrum from their dam within 2 h of birth. A presuckle blood sample was collected from each calf before colostrum feeding, with a follow-up blood sample collected from each calf 24 to 48 h after colostrum administration.

Serum and colostrum antibody titers for *Salmonella* Dublin were determined using an ELISA. The assay was performed by a commercial diagnostic laboratory (Animal Health Diagnostic Center, Cornell College of Veterinary Medicine, Ithaca, NY), and personnel performing the ELISA assays were not aware of group assignment for any of the animals. *Salmonella* Dublin titers were determined using a commercially available ELISA kit (PrioCHECK Salmonella Ab Bovine Dublin, Prionics Life Technologies, Schlieren, Switzerland) that has been validated for plasma, serum, and milk. This ELISA assay was developed in cooperation with the Danish Veterinary Institute and is based on the same principle as the Danish *Salmonella* Dublin ELISA that has been used extensively in previous studies (Nielsen and Erskbøll, 2004a,b, 2005). Results of the ELISA assays were reported as percent positivity.

Data are presented as mean ± standard error. Rectal-temperature and milk-production data along with serologic titers for cows and calves born to cows from each group were compared using one-way ANOVA. Values of *P* < 0.05 were considered significant. A statistical software package was used to conduct the data analysis (SAS, version 9.1; SAS Institute Inc., Cary, NC).

Rectal temperature did not significantly differ in any time point after vaccination as compared with baseline (prevaccination). Although milk production in both groups declined toward the end of the lactation period, vaccinated and control groups did not differ at any point during the 2-wk period where milk production was monitored. For the first 5 d following vaccination, daily milk production did not change from its baseline value (Table 1) in either group. A total of 59 of the 60 cows calved successfully and had colostrum samples collected (one cow from the control group aborted during the dry period and was removed from the study). *Salmonella* titers did not differ between groups of cows at the time of initial vaccination (Table 2). Cows in the vaccinated group had a significant (*P* = 0.007) increase

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group</th>
<th>Vaccinated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of cow (yr)</td>
<td>5.8 ± 2.4</td>
<td>5.4 ± 2.3</td>
</tr>
<tr>
<td>Lactation number</td>
<td>2.7 ± 1.4</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>DIM (current lactation)</td>
<td>341 ± 24</td>
<td>352 ± 29</td>
</tr>
<tr>
<td>305-d mature equivalent yield (kg)</td>
<td>10,400 ± 1,140</td>
<td>10,310 ± 1,020</td>
</tr>
<tr>
<td>Milk production—first day of study (kg)</td>
<td>19.3 ± 3.3</td>
<td>17.9 ± 3.1</td>
</tr>
</tbody>
</table>

*The data (means ± SD) were collected from farm records on the first day of the experimental period (3 wk before dry-off).*

Journal of Dairy Science Vol. 98 No. 4, 2015
in antibody titers at the time of calving as compared with unvaccinated cows. The colostrum collected from cows that had been vaccinated for *Salmonella* Dublin also had significantly (*P* = 0.03) higher antibody titers than did colostrum from unvaccinated cows, although *Salmonella* Dublin titers within cows varied considerably in the vaccinated group (Table 2).

Thirty calves born to control cows received colostrum in this study, and 29 calves born to vaccinated cows received colostrum. Two calves were dead at birth in the vaccinated group, but one cow had twins, which were both fed colostrum from the dam. One cow in the control group aborted during the dry period; however, another cow from the control group had twins, and both calves were included in the study. The stillbirth rates were, therefore, 2/30 (6.7%) in the vaccinated group and 1/30 (3.3%) in the unvaccinated group. Precolostrum *Salmonella* Dublin antibody titers did not differ between groups; however, calves that received colostrum from vaccinated cows had significantly (*P* < 0.001) higher *Salmonella* Dublin titers as compared with calves born to unvaccinated cows (Table 3).

This study indicates that the vaccination of late-gestation cattle with a commercially available *Salmonella* Dublin 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 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 *Salmonella* Typhimurium vaccine showed a reduction in fecal shedding of *Salmonella* after challenge (Royal et al., 1968). In another study, a group of dairy cows were vaccinated 2 and 7 wk before calving with a killed *Salmonella* Typhimurium vaccine, and another group was left unvaccinated (Jones et al., 1988). Calves were divided into several groups—those allowed to nurse colostrum from a vaccinated dam for 48 h 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 h and then fed colostrum from an unvaccinated cow throughout the study (VC/NM); calves that suckled an unvaccinated dam for 48 h and then were fed colostrum from a vaccinated cow after d 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 *Salmonella* Typhimurium on d 5. Death rates were significantly reduced in calves 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 with 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 *Salmonella* Typhimurium or *Salmonella* Dublin antibodies derived from chicken egg yolks was able to offer dose-dependent protection against an experimental challenge at 4 d of age (Yokoyama et al., 1998). All calves that were challenged with *Salmonella* but did not receive the egg-yolk antibodies died.

### Table 2. *Salmonella* Dublin antibody titers in cows and colostrum from cows that were vaccinated twice with *Salmonella* Dublin (vaccinated group) in late gestation as compared with cows that were not vaccinated (control group)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group</th>
<th>Vaccinated group</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vaccination (3 wk before dry-off)</td>
<td>−4.2 ± 1.5</td>
<td>−6.9 ± 0.9</td>
<td>0.68</td>
</tr>
<tr>
<td>After vaccination (at calving)</td>
<td>−9.4 ± 1.1</td>
<td>40.3 ± 9.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Colostrum</td>
<td>−17.2 ± 0.4</td>
<td>14.8 ± 7.6</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^1\)Data are presented as percent positivity.

### Table 3. *Salmonella* Dublin antibody titers in calves that received colostrum from cows that had been vaccinated twice with *Salmonella* Dublin (vaccinated group) in late gestation as compared with cows that were not vaccinated (control group)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group</th>
<th>Vaccinated group</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves (before colostrum)</td>
<td>−12.1 ± 0.4</td>
<td>−13.8 ± 0.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Calves (after colostrum)</td>
<td>−3.2 ± 1.2</td>
<td>88.5 ± 8.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\)Data are presented as percentage positivity.
In calves that received a low dose of antibodies, death rate was reduced from the control group but was still well over 50%; however, calves that received the higher titer of antibodies had fever and diarrhea but no deaths. Additional work done in sheep also demonstrated some protection associated with acquisition of passive immunity (Mukkur et al., 1998). Ewes were immunized 4 to 5 wk before lambing with an aro minus Salmonella Typhimurium vaccine, and their lambs were challenged with Salmonella Typhimurium orally at 2, 4, and 7 d of age. Higher Salmonella antibody titers were found in the colostrum from vaccinated ewes as compared with a group of unvaccinated ewes. In addition, lambs born to vaccinated ewes had a shorter duration of Salmonella shedding after challenge as compared with lambs born to unvaccinated ewes. The authors concluded that vaccination of the pregnant ewe conferred significant protection against experimental Salmonella challenge.

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 (Mohler et al., 2009). For example, the vaccine used in this study was an aromatic amino acid (aro) auxotroph Salmonella Dublin mutant that was given to calves beginning at 2 wk of age. Experimental studies with aro minus vaccines have demonstrated good protection against several different Salmonella serovars when calves were challenged within 3 wk after vaccination (Smith et al., 1984a,b). 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.

ACKNOWLEDGMENTS

This study was funded by Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO). The authors acknowledge Anthony Chesnut and Alli Davis of the North Carolina State University Dairy Educational Unit (Raleigh) for technical support.

REFERENCES


