Efficacy of oral administration of a modified-live
Salmonella Dublin vaccine in calves

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Objective—To determine the efficacy a modified-live Salmonella Dublin vaccine administered PO in an extralabel manner in the prevention of diseases associated with Salmonella Dublin infection.

Design—Randomized clinical trial.

Animals—288 preweaned Holstein dairy calves on a commercial dairy farm.

Procedures—Calves were orally administered either 2 mL of a commercially available, modified-live Salmonella Dublin vaccine (n = 140) or a placebo (148) at 3 and 10 days of age. Signs of diarrhea and depression were recorded daily. Weight gain between 3 days of age and time of weaning was measured. Fecal samples from clinically depressed or diarrheic calves and fresh tissues samples from calves that died were submitted for bacterial culture of Salmonella organisms.

Results—Salmonella organisms were isolated from samples of 1.4% (2/140) and 3.4% (5/148) of calves receiving the vaccine and placebo, respectively. Additionally, 57.1% (80/140) and 60.1% (89/148) of the vaccinated and control calves, respectively, had at least 1 day with an abnormal fecal score. Calves receiving the vaccine and placebo were not significantly different in terms of overall morbidity rate, Salmonella-specific morbidity rate, or average daily gain. Adverse reactions related to administration of the vaccine were not seen. The attenuated vaccine strain was not isolated from any fecal or tissue samples.

Conclusions and Clinical Relevance—This method of vaccination was safe in young Holstein calves, although it was not effective in reducing the incidence of disease or improving weight gain on this farm. However, the power of this study was limited by a low incidence of clinical salmonellosis. (J Am Vet Med Assoc 2011;238:1184–1190)

Livestock production is a major reservoir for Salmonella infections in humans. The CDC estimates that beef and dairy products are responsible for 10% of all human Salmonella clinical cases from outbreaks in which the vehicle of transmission is known.1 In addition, salmonellosis has an important influence on the production and welfare of dairy cattle. Modern calf-raising operations, which house large populations of susceptible neonates, experience high morbidity and mortality rates caused by outbreaks of salmonellosis. Serotypes most commonly isolated from clinical cases include Salmonella Typhimurium, Salmonella Newport, and Salmonella Dublin. Salmonella Dublin, the only cattle-adapted serotype, causes severe outbreaks of diarrhea and pneumonia in calf-raising units and is unique in its ability to produce chronically, subclinically infected carriers of the organism.2 Septicemia and pneumonia are frequent sequela to infection and can occur in the absence of clinical signs of enteritis. Treatment of calves infected with Salmonella Dublin is difficult. Its resistance to antimicrobial treatment increases production and welfare losses because of the chronicity and severity of the disease. Seventy percent of Salmonella Dublin isolates submitted to the Washington Animal Disease Diagnostic Laboratory by practicing veterinarians were resistant to >1 antimicrobial. Moreover, 25% of these isolates were resistant to ceftazidime, a cephalosporin important for treating human infections.3 Increasing cephalosporin resistance in Salmonella organisms was cited as a factor for a withdrawn FDA decision to prohibit the extralabel use of cephalosporins in food animals.4 The limitations and potential human health risks of antimicrobial treatment for infections caused by Salmonella Dublin highlight the need for effective preventative measures.

While research has improved our knowledge of risk factors for the transmission of Salmonella organisms, control of the disease remains difficult.5 The frequency of movement of people, animals, and equipment on modern commercial dairies and calf ranches makes exposure of young animals to Salmonella organisms difficult to prevent. In addition, newborn calves may be exposed to Salmonella Dublin through the shedding in colostrum or milk by a carrier dam.6 Improving resistance of animals to infection through vaccination would be a valuable tool because complete elimination of exposure is impossible on most commercial farms.

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The limited efficacy of commercially available killed bacterin or autogenously killed bacterin is due in part to the variety in antigenic composition of Salmonella organisms. In addition, most killed vaccines cause seroconversion but fail to stimulate an effective cell-mediated immune response. Both a humoral and a cell-mediated immune response are required for lasting protection against Salmonella infections. In contrast to a killed bacterin, modified-live Salmonella vaccines have been shown to stimulate both humoral and cell-mediated immune responses. Comparative vaccine trials have shown that modified-live Salmonella vaccines provide superior protection in both experimental and field settings.

Subcutaneous or IM injection of gram-negative bacterin can cause adverse reactions associated with impurities or endotoxins. These effects may be avoided with oral administration. Furthermore, while injection of vaccines may induce a systemic immune response, it often fails to trigger the key local immune mediators and agents that would prevent initial colonization of pathogens. With few exceptions, parenteral vaccines do not trigger the production of secretory IgA at mucosal surfaces. Villarreal-Ramos et al., using a modified-live Salmonella vaccine, found that oral but not SC immunization induced bovine serum IgA antibodies against Salmonella antigens. Oral vaccination is a promising alternative to parenteral administration. Examples of effective mucosal bovine vaccines include vaccines against bovine herpesvirus-1, rotavirus, and coronavirus.

The commercially available modified-live Salmonella Dublin vaccine is a genetically altered, aromatic ring–dependent (aro) Salmonella Dublin vaccine, which is labeled for SC administration in calves that are 2 weeks of age and older. A previous field trial in calves demonstrated efficacy of use of an IM route. Oral administration of aro vaccines has been effective in experimental conditions and has been shown to induce a cellular immune response. Commercial calf operations use this extralabel route of administration of the modified-live Salmonella Dublin vaccine to control outbreaks of salmonellosis, especially Salmonella Dublin, and avoid potential adverse effects associated with the parenteral route of administration. However, no field clinical trials have been performed to test the effectiveness of oral administration of this vaccine.

The purpose of the clinical trial reported here was to determine the efficacy of oral administration of a modified-live Salmonella Dublin vaccine in the prevention of diseases associated with Salmonella Dublin. Our hypothesis was that oral administration of a modified-live Salmonella Dublin vaccine to preweaned Holstein calves would result in improved growth rates and decreased morbidity and death caused by diseases associated with Salmonella Dublin. The clinical trial was conducted on a commercial calf-raising unit following the vaccine or control group at 3 days of age. A comparison of disease numbers to the vaccine or control group. A farm veterinarian and client considered the novel route of administration as a potential means to improve efficacy and decrease adverse effects without breaching any laws or standards of practice.

**Population description**—The animals used in this study were preweaned Holstein heifer and bull calves on a conventional dairy farm in Michigan that raised approximately 450 preweaned calves year-round in 6 calf barns with either forced or natural ventilation designs. Individual calf stalls in these barns allowed limited physical contact. Calves were fed a milk replacer with a 20% fat and 20% protein concentration twice daily, with calf starter offered free choice beginning at 3 days of age. At 6 weeks of age, calves were fed milk replacer once daily, and at 7 weeks of age, calves were no longer offered milk replacer. There were no antimicrobials included in the milk replacer or calf starter during the time of this study. Salmonella Dublin was isolated from fecal and necropsy specimens collected during an outbreak of diarrhea and pneumonia that lasted from January through summer 2008.

**Criteria for inclusion**—All calves born on the farm between September 26 and November 9, 2008, and intended to be kept on the farm to be raised as replacement bulls or heifers were enrolled into the study. Calves with substantial congenital abnormalities or that were sick at the time of enrollment were excluded from the study.

**Planned interventions and their timing**—The vaccine protocol used in this study was designed to be similar to the protocol being used for oral administration of this vaccine on other Midwestern dairy farms. Enrolled calves received either the vaccine or placebo at 3 and 10 days of age. Calves in the vaccine group were orally administered a mixture containing 2 mL of the commercial preparation and 18 mL of an antacid solution containing 5% sodium bicarbonate, 5% magnesium carbonate, and 5% magnesium trisilicate. The purpose of the antacid solution was to improve passage of the attenuated bacteria through the abomasum into the small intestine, where stimulation of an effective immune response is more likely.

Calves in the placebo group received 20 mL of the antacid solution orally. The calves were allowed to suckle the syringe. If they were unwilling to suckle the syringe, the solution was administered slowly into the pharynx.

**Sample size calculation**—The sample size was calculated a priori to detect a difference in the overall morbidity rate. Morbidity rates of 0.20 and 0.33 were used for the vaccine and control group, respectively. With $\beta = 0.80$ and $\sigma = 0.05$, the total number of calves required to show a significant difference between the 2 groups was calculated to be 295.

**Assignment**—Calves were randomly assigned to the vaccine or control group at 3 days of age. A computer program was used to randomly assign identification numbers to the vaccine or control group. A farm manager uninvolved with calf barn activities used these numbers to assign calves to the vaccine or control group.
randomization sheets to prepare the syringes daily. Syringes were refrigerated until administration.

**Blinding**—All farm staff and veterinarians involved with calf management, calf diagnoses and treatments, vaccine and placebo administration, data collection, and data analysis were blinded to group assignments. Syringes containing vaccine were indistinguishable from those containing placebo.

**Outcomes**—Planned outcomes included overall morbidity, *Salmonella* morbidity, growth rate, and mortality rate during the preweaning period. Overall morbidity was defined as the proportion of calves with at least 1 day of an abnormal fecal (≥ 2) or depression score (≥ 1). *Salmonella* morbidity was defined as the proportion of calves in the group that met the case definition requirements for salmonellosis. Other outcomes considered were treatment morbidity and body weight at weaning. Treatment morbidity was defined as the proportion of calves that received at least 1 antimicrobial treatment prior to weaning by farm management, regardless of diagnoses. These 2 post hoc outcomes were included to increase the sensitivity for detecting differences between the 2 groups of calves.

**Case definition**—Fecal samples were collected from all calves with a fecal score of ≥ 3. Additionally, fecal samples were collected from any calf with a depression score of ≥ 2 and a rectal temperature > 102.9°F (39.4°C). Any calf that went on to have positive bacterial culture results for *Salmonella* organisms was considered to have met the prespecified requirements for a case of salmonellosis. This broad case definition was used to capture calves with septicaemia or pneumonia caused by *Salmonella* Dublin that were not showing clinical signs of enteritis. Sick calves received medical treatment according to farm protocol and at the discretion of the herd manager and farm veterinarians.

**Measurements**—To assess failure of passive transfer, a serum sample was collected at 3 days of age for total protein measurement. Fecal and depression scores were assigned daily by a veterinarian to calves from 3 days of age until time of weaning. Fecal scores were assigned as follows: 1 = formed; 2 = semiformal; 3 = loose but enough consistency to stay on top of the bedding; and 4 = manure that is watery and sifts through the bedding. A depression scoring system was used as follows: 1 = normal; 2 = mild depression with sunken eyes and skin tenting lasting 2 to 6 seconds; 3 = very depressed, reluctant to get up, and a skin tent lasting > 6 seconds; and 4 = a calf whose skin would not flatten when tented and would not stand. For comparison of growth weights, heart girth circumference was measured at 3 days of age and again between 39 and 51 days of age by use of a heart girth weight tape. Chest circumference in centimeters was used to estimate body weight in kilograms, and the average daily gain was computed from the measurements taken at 3 days of age and weaning.

Liver, spleen, lung, and colon tissues were collected from calves that died during the study; these tissues were kept frozen at −20°C (−4°F). At the end of the study, the tissues were collectively submitted to the Diagnostic Center for Population and Animal Health at Michigan State University for isolation and identification of *Salmonella* organisms.

**Laboratory procedures**—Fecal samples were collected and then processed on the same day. Tetraionate broth was added directly to the fecal samples so as to achieve a 1:10 dilution of sample and enrichment broth. This mixture was then incubated for 48 hours at 37°C. The enriched sample was streaked onto xylose lysine deoxycholate agar and incubated for 24 hours at 37°C. One oxidase-negative colony (red or yellow with black center) was inoculated into a tube with layered media designed for the differentiation of enteric pathogens. Layers of the media consisted of triple sugar iron agar, lysine iron agar, and urea agar. Samples were considered positive if they were urease and lysine decarboxylase and hydrogen sulfide positive. Isolates identified as *Salmonella* spp were suspended in tryptase soy broth, 0.5 mL of the suspension was added to 0.5 mL of 65% glycerol solution, and the mixture was frozen at −20°C. Confirmation of *Salmonella* spp and serotype identification of *Salmonella* organisms isolated from fecal samples were performed at the Diagnostic Center for Population and Animal Health at Michigan State University.

For serum total protein measurements, blood was collected from the jugular vein of calves into serum separator tubes. The blood was allowed to clot and then centrifuged for 15 minutes at 1,170 × g. Serum was harvested, and the total protein concentration was determined by use of a standard refractometer.

**Statistical analysis**—All statistical procedures were carried out by use of standard statistical software. Overall morbidity, *Salmonella* morbidity, and treatment morbidity were compared by use of a Fisher exact test. Average daily gain and mean body weight at weaning of the vaccine and control groups were compared by use of a 2-sample *t* test. It had been planned to model the length of survival of calves in both groups by use of the Cox proportional hazards model. However, because of the low frequency of deaths and the lack of numeric difference in the number of deaths in both groups, the modeling was not included in the final statistical analysis. Statistical analyses were completed on an intention-to-treat basis. A value of α = 0.05 was used as a critical value to show significance of differences between the vaccine and placebo groups.

**Results**

Three hundred six eligible replacement calves were born during the time frame of the study. Eighteen of those animals were not enrolled into the study at 3 days of age. Reasons for exclusion included death prior to enrollment (n = 3), congenital disease (4), or inadvertent omission (11). One hundred forty calves were enrolled into the vaccine group, and 148 calves were enrolled into the placebo group. One calf in the placebo group was lost to follow-up. The analysis included all calves that were enrolled into the study. Calves in the vaccine and placebo group did not differ by barn location, sex, body weight at enrollment, serum total protein concentration, or age at weaning (Table 1). Pas-
sive transfer of maternal immunoglobulins in calves appeared to be adequate, as only 13.8% (39/283) of calves had serum total protein concentrations < 5.5 mg/mL. During the follow-up period, 56 of 140 (40%) calves in the vaccine group and 64 of 148 (43.24%) calves in the control group had at least 1 day with an abnormal depression score. There were 80 of 140 (57.1%) vaccinated calves and 89 of 148 (60.1%) control calves that had at least 1 day with an abnormal fecal score. Of the fecal samples collected, 3.7% (6/161) were positive on culture for Salmonella organisms. Six of the isolates were identified as Salmonella Litchfield, and one of the isolates was identified as Salmonella Agona. Twenty-eight percent (82/288) of the calves were isolated into the study died prior to weaning. No Salmonella organism was isolated from tissues collected at necropsy. The incidence of adverse effects attributable to vaccination was low. Only 1 calf had signs of diarrhea in the 48 hours following the first vaccination at 3 days of age. None of the calves were noted to have signs of depression in the 48 hours following the first vaccination. The occurrence of diarrhea or signs of depression in the 48 hours following the second vaccination at 10 days of age was similar between vaccine and placebo groups.

Table 1—Baseline descriptors for calves administered a modified-live Salmonella Dublin vaccine (vaccine group; n = 140) or a placebo (control group; 148).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vaccine group (95% confidence interval)</th>
<th>Control group (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housing (% in forced ventilation barn)</td>
<td>35.07 (27.0–43.2)</td>
<td>40.14 (32.0–48.7)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>27.50 (20.0–35.0)</td>
<td>26.67 (19.6–33.74)</td>
</tr>
<tr>
<td>Mean body weight at enrollment (kg)</td>
<td>42.05 (41.15–42.97)</td>
<td>42.96 (42.01–43.90)</td>
</tr>
<tr>
<td>Mean serum total protein (mg/dL)</td>
<td>6.08 (5.98–6.18)</td>
<td>6.01 (5.93–6.10)</td>
</tr>
<tr>
<td>Mean age at weaning (d)</td>
<td>46.76 (46.12–47.32)</td>
<td>46.80 (46.20–47.21)</td>
</tr>
</tbody>
</table>

Overall morbidity, specific morbidity, and treatment morbidity—Similar proportions of calves in the vaccine and control groups had at least 1 day of an abnormal fecal score or depression score (P = 0.61; Table 2). Additionally, the proportion of calves meeting the case definition (positive bacterial culture result for Salmonella organisms) did not significantly (P = 0.30) differ by treatment group. The treatment rate (proportion of calves requiring at least 1 antimicrobial treatment) was not significantly (P = 0.85) different between calves in the vaccine and control groups.

Overall mortality rate—Four calves in the vaccine group and 5 calves in the control group died during the study period (P = 0.83; Table 2). Salmonella organisms were not isolated from any harvested tissues.

Weight gain—The mean age at weaning was similar for calves in the vaccine (46.19 days) and control (46.20 days) groups. Calves gained a mean of 0.46 kg/d (1.012 lb/d) between 3 days and the time of weaning (Table 3). Average daily gain and mean body weight at weaning for vaccinated or unvaccinated calves were not significantly (P = 0.12) different.

Discussion

In this study, the use a modified-live Salmonella Dublin vaccine administered PO in an extralabel manner to preweaned calves did not have a measurable influence on the incidence of disease or growth prior to weaning. Importantly, calves receiving the vaccine did not have observable or measurable adverse effects. Furthermore, shedding of the attenuated vaccine Salmonella strain was not isolated from samples of diarrheic calves or calves with signs of depression throughout the study.

Food animal veterinarians are integral to ensure the proper use of drugs and vaccines on dairy farms. Guidelines for veterinarians regarding the extralabel use of antimicrobials and other pharmaceuticals have been outlined in the AMDUCA. Vaccines, however, are not subject to regulation by the FDA. Rather, the USDA substantiates the safety and quality of vaccines manufactured for livestock. Labels on USDA-licensed

Table 2—Number (%) of calves in the vaccine (n = 140) and control (148) groups with various morbidity outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vaccine group</th>
<th>Control group</th>
<th>Risk ratio (95% confidence interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall morbidity</td>
<td>75 (53.57)</td>
<td>86 (58.10)</td>
<td>0.95 (0.77–1.16)</td>
<td>0.61</td>
</tr>
<tr>
<td>Abnormal depression score</td>
<td>56 (40.0)</td>
<td>64 (43.24)</td>
<td>0.91 (0.69–1.19)</td>
<td>0.46</td>
</tr>
<tr>
<td>Abnormal fecal score</td>
<td>89 (57.14)</td>
<td>89 (56.14)</td>
<td>0.83 (0.77–1.13)</td>
<td>0.47</td>
</tr>
<tr>
<td>Salmonella morbidity</td>
<td>2 (1.43)</td>
<td>5 (3.38)</td>
<td>0.43 (0.09–2.20)</td>
<td>0.30</td>
</tr>
<tr>
<td>Treatment morbidity</td>
<td>41 (29.29)</td>
<td>41 (27.70)</td>
<td>1.04 (0.072–1.49)</td>
<td>0.85</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>4 (2.85)</td>
<td>5 (3.38)</td>
<td>0.87 (0.24–3.17)</td>
<td>0.83</td>
</tr>
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Table 3—Mean ± SD body weight outcomes for calves in the vaccine (n = 138) and control (140) groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vaccine group</th>
<th>Control group</th>
<th>Difference (95% confidence interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (kg/d)</td>
<td>0.45 ± 0.17</td>
<td>0.48 ± 0.26</td>
<td>–0.04 (–0.09 to 0.01)</td>
<td>0.12</td>
</tr>
<tr>
<td>Body weight at weaning (kg)</td>
<td>63.58 ± 8.30</td>
<td>65.0 ± 8.53</td>
<td>–1.42 (–3.38 to 0.37)</td>
<td>0.11</td>
</tr>
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Salmonella vaccine at 2 weeks of age were protected against an oral infection of Salmonella organisms, and reduced colonization of internal organs by Salmonella organisms. The historical lack of efficacy of a killed Salmonella bacterin in the prevention of salmonellosis in cattle has led to research to investigate alternatives. Modified-live vaccines have shown positive results. Specific gene deletions have been used to render otherwise virulent Salmonella organisms avirulent, while retaining immunogenicity. Aromatic-dependent (aro) Salmonella organisms have a gene deletion that prevents the synthesis of p-aminobenzoic acid, an aromatic compound required for the synthesis of folate. Because p-aminobenzoic acid is absent in mammalian tissues, aro Salmonella organisms are unable to synthesize or assimilate folate from surrounding tissues. The resultant organism has reduced virulence but retains the ability to stimulate a strong cell-mediated immune response in the host.

Research trials examining parenteral and oral vaccination with aro Salmonella vaccines have shown promise. In oral challenge experiments, IM vaccination by use of aro Salmonella organisms has been successful in protecting calves from virulent Salmonella organisms. Furthermore, a large field clinical trial on a commercial calf-raising facility showed significantly fewer deaths associated with Salmonella Dublin in calves administered an aro Salmonella Dublin vaccine IM.

Oral administration of aro Salmonella vaccines has been used successfully in experimental situations. Calves receiving 1 dose of an aro Salmonella Dublin vaccine at 2 weeks of age were protected against an oral Salmonella Dublin challenge 25 days later. Additionally, 1-week-old calves receiving a single inoculation of an aro Salmonella Typhimurium had protection from an oral Salmonella challenge. Other similar experimental studies that used aro Salmonella vaccines have shown positive results.

Effective presentation of attenuated Salmonella organisms to the lymphatic tissues in the gastrointestinal tract is problematic. Gerds et al state that “mucosal vaccination is proving to be one of the greatest challenges in modern vaccine development.” In order to induce an immune response, oral vaccines in cattle must overcome a plethora of innate and nonspecific immune mechanisms present at the mucosal surface in the gastrointestinal tract. Even so, live, genetically altered aro Salmonella organisms, with a tropism for intestinal cells, are a strong candidate to overcome these difficulties and induce protection from a virulent challenge. Specifics of this study protocol, including the dosage of organisms and age at vaccination, may have influenced the ability of the vaccine to protect calves on a commercial farm against natural exposure. The 2-mL vaccine volume used in this study was chosen because it follows the protocol that is commonly used on Midwestern dairy farms. However, the dosage of organisms required for an effective immune response after oral administration may be substantially higher. Successful experimental studies most commonly used oral doses of 1 X 10^9 Salmonella bacteria to induce protection. Smith et al, using an orally administered aro Salmonella vaccine, found that a dose of 1 X 10^10 organisms, effectively protected calves from a virulent challenge. The concentration of organisms in the commercial vaccine used in this study is several logarithms lower than the concentration used in those experimental trials. Therefore, the volume of commercial vaccine required to orally administer 1 X 10^10 organisms would not be feasible.

Calves enrolled in this clinical trial were administered the vaccine between 3 and 10 days of age. The timing of vaccination for this trial was chosen to sufficiently induce an immune response prior to the age of clinical onset at this farm and to stay consistent with the field protocol. In this study, it is unknown if maternal antibodies interfered with the induction of an immune response. For calves enrolled in the study, the last colostrum feeding was at 4 hours of age. Vaccination of calves in the face of maternal antibodies has been shown to be effective for some types of mucosal vaccines, and effective protection by use of aro Salmonella vaccines has been induced via oral vaccination in calves as young as 7 days of age. This supports the double vaccination protocol that is used in the field and was tested in this clinical trial.

Parenteral administration of a gram-negative bacterin is known to cause endotoxin-mediated adverse reactions, including dyspnea and death. Oral administration of aro Salmonella vaccines has caused mild clinical signs, including fever and diarrhea. Importantly, the incidence of adverse effects in this study was low. No adverse effects suggestive of anaphylaxis were observed immediately following oral administration of the vaccine. No vaccinated calves died in the 2 days following the first administration of the vaccine at 3 days of age. Only 1 vaccinated calf was observed to have diarrhea during the 2 days following the first vaccination, and no calves were observed to have signs of depression during the 2 days following the first vaccination. Fifteen calves in the vaccine group were observed to have diarrhea or signs of depression within the 2 days following the second vaccination at 10 days of age, but this was similar to the number in the placebo group (n = 12).

In experimental trials, oral administration of Salmonella vaccines has resulted in fecal shedding of the attenuated organism for up to 1 week after vaccination. In this study, the attenuated Salmonella organism was not isolated from any of the 40 fecal samples collected from vaccinated calves within 1 week after vaccine administration. Salmonella organisms were not isolated from any fresh tissues collected from vaccinated calves that died during the study. The calf operation used in this clinical trial experienced an outbreak of pneumonia and diarrhea from...
January 2008 to the commencement of this study. *Salmonella* Dublin was isolated from fecal samples, tracheal swabs, and necropsy tissues in January, March, June, and July 2008. Of the 29 diagnostic samples collected from the calves at this farm during the 8 months prior to the clinical trial, 11 were positive for *Salmonella* Dublin. Enrollment for this clinical trial began in September 2008. Although the trial was initiated following an outbreak of salmonellosis, *Salmonella* organisms were isolated from only 7 fecal samples. Despite the recent outbreak, none of these isolates were identified as *Salmonella* Dublin. The apparent decline in incidence of *Salmonella* Dublin and increase in other serotypes between the start of the herd outbreak and end of the clinical trial are interesting. The intensity and duration of salmonellosis outbreaks are dependent on many factors, including the level of herd immunity, virulence of the pathogen, and number of opportunities for direct and indirect transmission.38 Outbreaks in calf barns can be especially severe because of the constant introduction of susceptible neonates. Immune function in neonatal calves is largely dependent upon the successful passive transfer of antibodies via colostrum. Although it was not confirmed, introduction of the organism into the cow herd may have resulted in passive transfer of antibodies specific to the *Salmonella* Dublin strain. Induction of herd immunity in this population of calves may have been responsible for the low shedding of *Salmonella* organisms and low mortality rate during the course of the trial. Additionally, management practices were implemented that were designed to interrupt the transmission of *Salmonella* organisms among calves. Special attention was paid to passive transfer of immunoglobulins, cleaning and disinfection of calf barns, and decreasing the amount of people and equipment traffic between calf barns and near the adult cow herd. Moreover, seasonal variations in the shedding of *Salmonella* organisms in cattle have been documented. In 1 large study39 of *Salmonella* shedding in dairy cattle, cows and calves were 2.5 times as likely to shed *Salmonella* organisms during summer, compared with during winter. Because the present study began in fall, the decrease in incidence of salmonellosis may have been attributable to seasonal effects. Regardless of the cause, the decline in clinical disease and shedding limited the ability of this trial to demonstrate the efficacy of the vaccine. Importantly, this trial showed that any improvements in health that occurred during the time frame of the study were caused by factors other than the vaccine administration.

As a diagnostic test, bacterial culture of fecal samples for *Salmonella* organisms suffers from low sensitivity. *Salmonella* Dublin, in particular, is fastidious in nature and is shed intermittently. The sensitivity for detection of *Salmonella* Dublin in fecal samples has been estimated to be between 6% and 14%.32 The characteristics of this test likely resulted in misclassification error, further reducing the power of this study to demonstrate a difference in *Salmonella* shedding in vaccinated and control calves. In conclusion, the results of this study demonstrate that this method of vaccination can be used safely in calves as young as 3 days of age. The vaccine strain was not isolated from any fecal samples or fresh tissues collected from calves with diarrhea or signs of depression. Implementation of this vaccination protocol following an outbreak of *Salmonella* Dublin on this commercial calf-raising operation had no measurable effect on overall calf morbidity, mortality rate, or growth. However, it is important to recognize that this trial was implemented 8 months into a clinical investigation, when the incidence had decreased to lower levels, possibly because of management and herd immunity changes. With the data from this trial, unnecessary continued vaccine use and associated costs were avoided.

The frequency of use of this extralabel method of vaccination on commercial calf-raising operations follows a demand from producers and veterinarians for effective preventative measures to address outbreaks of salmonellosis in young calves, especially *Salmonella* Dublin. Despite a plethora of commercially available *Salmonella* vaccines, few field clinical trials have been performed to demonstrate effective prevention of disease or improved performance. Oral administration of attenuated *Salmonella* vaccines remains a promising tool for the prevention of salmonellosis in young calves. However, development of vaccines specifically designed for oral use, together with additional experimental studies to identify the appropriate protocol, is needed. Field clinical trials conducted on commercial calf-raising operations will ultimately be necessary to demonstrate the effectiveness of these vaccines.

### References

Evaluation of N-terminal pro-B-type natriuretic peptide as a diagnostic marker of various stages of cardiomyopathy in Doberman Pinschers

Gerhard Wess et al

Objective—To evaluate the diagnostic value of plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations in Doberman Pinschers in various stages of dilated cardiomyopathy (DCM).

Animals—328 Doberman Pinschers.

Procedures—Staging of DCM was determined via analysis of results of physical examinations, 24-hour ambulatory ECG (Holter) recordings, and echocardiographic evaluations. Plasma samples for NT-proBNP assays were obtained at each examination. Concentrations of NT-proBNP were measured in 337 samples obtained from 196 healthy Doberman Pinschers (control dogs) and in 195 samples obtained from 132 Doberman Pinschers in various stages of DCM. These included dogs that had ventricular premature contractions (VPCs; 79 samples), echocardiographic changes (23 samples), or both (51 samples). 16 samples were from dogs with overt DCM, and 26 were from dogs that were considered normal during initial examination, but developed DCM within 1.5 years after this assessment. Receiver operating characteristic curves were analyzed to determine sensitivity and specificity of NT-proBNP concentrations for detection of DCM.

Results—NT-proBNP values in dogs that had or developed DCM were significantly higher than those of control dogs. Sensitivity and specificity of NT-proBNP concentrations (cutoff value, > 500 pmol/L) to detect all stages of DCM were 81.1% and 75.0%, respectively; sensitivity was 90.0% and specificity was 75.0% to predict echocardiographic changes. Specificity to detect echocardiographic changes was 90.4% at > 550 pmol/L.

Conclusions and Clinical Relevance—Plasma concentrations of NT-proBNP were increased in dogs with DCM and in apparently healthy dogs that developed DCM within 1.5 years after samples were obtained, compared with concentrations in control dogs. (Am J Vet Res 2011;72:642–649)

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