Effect of changes in number of doses and anatomic location for administration of an *Escherichia coli* bacterin on serum IgG1 and IgG2 concentrations in dairy cows

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**Objective**—To determine effects of injection site on antibody response to J5 *Escherichia coli* bacterin.

**Animals**—28 adult Holstein cows.

**Procedures**—Cows were randomly assigned as control cattle (n = 4 cows), not administered J5 *E coli* bacterin; 3X (8), administered 3 doses of bacterin SC in the left side of the neck; 5X/N (8), administered 5 doses of bacterin SC in the left side of the neck; or 5X/SR (8), administered 5 doses of bacterin SC sequentially in the left side of the neck, right side of the thorax, left side of the thorax, and left side of the neck. Blood samples were collected from the cows to determine anti-J5 *E coli* IgG1 and IgG2 concentrations.

**Results**—Vaccinated cows had higher mean serum anti-J5 *E coli* IgG1 concentrations than did control cows. The 5X/N and 5X/SR cows had higher mean serum anti-J5 *E coli* IgG1 concentrations than did 3X cows. Additionally, 5X/SR cows had higher mean serum anti-J5 *E coli* IgG1 concentrations than did 5X/N cows. Vaccinated cows had higher mean serum anti-J5 *E coli* IgG2 concentrations than did control cows. The 5X/N and 5X/SR cows had higher mean serum anti-J5 *E coli* IgG2 concentrations than did 3X cows. The 5X/SR cows had higher mean serum anti-J5 *E coli* IgG2 concentrations than did all other groups at 84 days after the fifth vaccination.

**Conclusions and Clinical Relevance**—Sequential doses of core-antigen bacterins administered at different anatomic locations may improve antibody response in dairy cattle. (*Am J Vet Res* 2010;71:120–124)

*Escherichia coli* bacterins are used extensively in cattle to enhance immune resistance against gram-negative bacteria, including mastitis-causing coliforms. Commercial J5 *E coli* bacterins are formulated with a mutant strain of *E coli* O111:B4 (Rc mutant) that lacks the O antigen capsule of the cell wall but that has the core lipopolysaccharide and lipid A antigens intact. These core antigens are highly conserved among gram-negative bacteria and elicit cross-reactive antibodies in cows vaccinated against *J5 E coli*.3–5

Initial field trials determined that 3 vaccinations during the nonlactating period and early lactation reduced the rate of clinical mastitis caused by gram-negative pathogens during the first 3 months of lactation.6,7 A more recent study8 revealed that clinical mastitis in nonvaccinated cows was nearly 3 times as likely to result in culling or death as clinical mastitis in J5-vaccinated cows. Additionally, higher serum anti-J5 *E coli* IgG concentration was correlated with a lower incidence of clinical coliform mastitis.9 Investigators in other studies10-12 have determined that vaccination with J5 *E coli* bacterin increases concentrations of anti-J5 *E coli* immunoglobulins, especially G1 and G2 isotypes, in serum and milk, compared with concentrations in unvaccinated control cattle. However, the ability to reduce the severity of infection following intramammary challenge exposure with *E coli* has been inconclusive.10-13 Although vaccination with J5 *E coli* bacterin may decrease the severity of infections caused by coliform organisms, it does not reduce the overall rate of clinical mastitis.9 Additionally, cows may have a period with an increased incidence of severe coliform mastitis near peak lactation (60 to 150 days of lactation) despite administration of 3-dose regimens of J5 *E coli* bacterins.14 Gram-negative bacterins are regarded as weakly immunogenic in cattle because they elicit poor anamnestic IgG1 and IgG2 responses.15 In another study14...
conducted by our laboratory group, we determined that although administration of 6 doses of bacterin to lactating cows reduced the incidence of severe mastitis caused by coliform organisms and improved survival through 305 days of lactation, increases in serum IgG2 concentrations gained by administration in accordance with this vaccination schedule did not remain into the next lactation.

Despite less than ideal immune responses after vaccination with J5 E coli bacterins, little is known regarding the effect of changing injection sites throughout a vaccination regimen. The objective of the study reported here was to determine the effect of changing injection sites during a 5-dose vaccination schedule with J5 E coli bacterin on serum IgG1 and IgG2 concentrations in lactating dairy cattle.

**Materials and Methods**

**Animals**—Adult (≥ 2 lactations) Holstein cows (n = 28) were housed and cared for at the Kellogg Biological Station Dairy at Michigan State University. Cows were housed in groups, had unlimited access to water and feed, and were milked 3 times/d. Cows were enrolled at the end of a lactation when it was determined by the herd manager that they were healthy on the basis of results of a physical examination (appetite, rectal temperature, attitude, and lack of clinical mastitis). The study protocol was approved by the Michigan State University All-University Committee on Animal Use and Care.

Cows remained in the study throughout the nonlactating period and the first 21 weeks of the ensuing lactation. Because the intent was to compare antibody response with the bacterin in healthy cows throughout the entire study period, cows that were culled or died before study completion (n = 3 cows) were replaced. None of the 3 cows were removed because of mastitis or adverse reactions to the vaccine.

**Vaccination schedule**—Cows were randomly assigned to 1 of 4 treatment groups. The control group (n = 4 cows) was not administered J5 E coli bacterin. Group 3X (n = 8) was administered a 5-mL dose of a commercial gram-negative mastitis bacterin by SC injection 3 times (on the last day of lactation [approx 7 weeks before parturition], approx 3 weeks before parturition, and the first week after parturition [between 3 and 9 days after parturition]). All doses were administered SC in the left side of the neck. Group 5XN (n = 8) was administered 5 doses of bacterin (the same 3 doses as group 3X and 2 additional doses; the additional doses were administered 28 and 56 days after the third vaccination [5 weeks [between 31 and 37 days] and 9 weeks [between 57 and 65 days] after parturition, respectively]), and all doses were administered SC in the left side of the neck. Group 5XSNR (n = 8) also was administered 5 doses of bacterin at the same times relative to parturition as for group 5XN. However, the 5 doses in this group of cattle were administered SC at various anatomic locations (sequentially in the left side of the neck, right side of the neck, right side of the thorax, left side of the thorax, and again in the left side of the neck).

**Blood collection schedule**—Blood samples were collected from all cows at the same time points, regardless of vaccination group, and used to determine anti-J5 E coli IgG1 and IgG2 concentrations in serum. Samples were collected on the days of the first, second, and third vaccinations, 14 days after the third vaccination; 28 days after the third vaccination (ie, day of the fourth vaccination), 56 days after the third vaccination (ie, day of the fifth vaccination), 84 days after the third vaccination (ie, 28 days after the fifth vaccination), and 84 days after the fifth vaccination. Blood samples were collected from the coccygeal vein into 15-mL sterile glass tubes, allowed to clot overnight at 4°C, and then centrifuged at 1,500 X g for 15 minutes at 4°C. Serum was harvested and stored in 2-mL aliquots at −20°C until assayed by use of an ELISA.

**Preparation of J5 E coli whole-cell antigen for ELISA**—The J5 E coli whole-cell antigen was prepared as described elsewhere. Briefly, isolated colonies from pure cultures were used to inoculate trypticase soy broth, which was incubated with shaking at 120 rounds/min for 18 hours at 37°C. The bacterial culture was checked for purity; then 99% phenol was added, and the solution was shaken for 1 hour at 120 rounds/min at 37°C. The phenol-killed whole-cell bacteria were centrifuged at 1,000 X g for 12 minutes at 4°C; the pellet was washed twice and centrifuged in 500 mL of sterile saline (0.9% NaCl) solution. After the second centrifugation, the pellet was suspended in sterile PBS solution, which was added until we achieved an optical transmission of 13% (approx 1 X 10⁶ CFUs/mL) at 610 nm, as determined via a spectrophotometer. The whole-cell J5 antigen solution was stored in 15-mL aliquots at −20°C until used in the ELISA.

**ELISA to determine anti-J5 E coli IgG1 and IgG2 responses**—Serum anti-J5 E coli IgG1 and IgG2 response patterns were determined by use of an ELISA protocol reported elsewhere, as described by Tyler et al. The antigen was phenol-killed whole-cell J5 E coli, the detection antibody was horseradish peroxidase–conjugated sheep anti-bovine IgG1 or IgG2 (each diluted 1:25,000 in sample diluent), and the substrate was hydrogen peroxide-azino-bis-3-ethylbenzthiazoline sulfonic acid. Test sera were diluted in diluent (PBS solution [pH, 7.3] with 0.5% Tween 20) at a dilution of 1:400 for IgG1 and 1:200 for IgG2; sera were assayed in triplicate. The assay positive control sample was pooled sera from steers hyperimmunized by administration of J5 E coli bacterin, and the negative control sample was fetal bovine serum. Both positive and negative control samples were diluted at 1:400 (for IgG1) or 1:200 (for IgG2) and included in triplicate on every ELISA plate. Before sera were assayed by use of the ELISA, we tested serial titers with the positive test serum and determined the range of antibody response that was dependent on change in titer. The midpoint of the response curve was selected as the test sera dilution. Concentrations of IgG1- and IgG2-specific anti-J5 E coli antibodies in the test and control samples were recorded as the optical density as determined by dual-wavelength spectrophotometric analysis (405 nm for test and control samples, after normalization of the entire plate at 450 nm on the
basis of 3 blank wells containing only diluent) by use of an ELISA plate reader. Thus, normalized optical densities at 405 nm were the final data set for the statistical analysis.

Data analysis—All data analysis was conducted with a commercial statistical program. A repeated-measures design was used to compare optical densities of IgG1 and IgG2 between treatment groups at each time point. For all analyses, values of $P < 0.05$ were considered significant.

Results

Vaccination with the J5 E. coli bacterin resulted in significantly higher serum anti-J5 E. coli IgG1 concentrations in the 3X, 5XN, and 5XSR cows than in the unvaccinated control cows from 14 days after the third vaccination through 28 days after the fifth vaccination (Figure 1). Values for only the 5XSR group were significantly ($P = 0.001$) higher than those for the control cows at 84 days after the fifth vaccination. Administration of 5 doses of J5 E. coli bacterin (5XN and 5XSR groups) resulted in higher serum anti-J5 E. coli IgG1 concentrations than that in cows vaccinated with 3 doses (3X group). However, cows in the 5XN group had significantly ($P = 0.033$) higher concentrations than did cows in the 3X group only at 28 days after the fifth vaccination. Cows in the 5XSR group also had a significantly ($P = 0.01$) higher serum IgG1 concentration than did cows in the 3X group from 28 days after the third vaccination through 84 days after the fifth vaccination. Cows in the 5XSR group remained significantly ($P = 0.001$) higher than those in the unvaccinated control cows through 84 days after the fifth vaccination.

Similarly, vaccination with the J5 E. coli bacterin resulted in significantly higher serum anti-J5 E. coli IgG2 concentrations in the 3X, 5XN, and 5XSR cows than in the unvaccinated control cows (Figure 2). However, concentrations for the 3X group were significantly ($P = 0.027$) higher only at 56 days after the third vaccination, and concentrations for the 5XN group were significantly higher 28 days after the fourth and fifth vaccinations. Concentrations for the 5XSR group remained significantly ($P = 0.02$) higher than concentrations for the control cows from 14 days after the third vaccination through day 84 after the fifth vaccination. Administration of 5 doses of J5 E. coli bacterin resulted in significantly higher serum anti-J5 E. coli IgG2 concentrations, compared with that for cows vaccinated by administration of 3 doses, at 28 days after the fifth vaccination. However, the concentration for only the 5XSR group was significantly ($P = 0.012$) higher than the concentration for the 3X group at 84 days after the fifth vaccination. The 5XSR

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Figure 1—Mean ± SEM serum IgG1 concentration (reported as optical density [OD] at 405 nm) in 4 cows not administered a J5 E. coli bacterin (control group [white circles]), 8 cows vaccinated with 3 doses of bacterin administered SC in the left side of the neck (3X group [black diamonds]), 8 cows vaccinated with 5 doses of bacterin administered SC in the left side of the neck (5XN group [black triangles]), and 8 cows vaccinated with 5 doses of bacterin administered SC in the left side of the neck, right side of the thorax, left side of the thorax, and left side of the neck (5XSR group [black squares]). Blood samples were collected from all cows at the time of the first (V1; approx 7 weeks before parturition), second (V2; approx 3 weeks before parturition), third (V3; first week after parturition), fourth (V4; 5 weeks [between days 3 and 9 after parturition]), fifth (V5; 9 weeks [between 57 and 65 days after parturition]) vaccinations and 14 days after the third vaccination, 28 days after the fifth vaccination, and 84 days after the fifth vaccination. Day of parturition was designated as day 0. *Within a time point, mean values for groups with different letters differ significantly ($P < 0.05$).

Figure 2—Mean ± SEM serum IgG2 concentration (reported as OD at 405 nm) in 4 control cows not administered a J5 E. coli bacterin, 8 cows in the 3X group, 8 cows in the 5XN group, and 8 cows in the 5XSR group. See Figure 1 for remainder of key.
bacterin responded to the J5 E coli IgG1 and IgG2 concentrations were selected as indicators of antibody response to vaccination in this study. In other studies, it has been suggested that serum IgM concentrations also increase in response to J5 E coli administration. However, we focused on IgG1 and IgG2 because results of 1 study suggest that changes in these isotypes indicate a potentially more mature immunoglobulin response. Additionally, IgG2 plays a critical role in neutrophil phagocytosis and has been associated with increased recognition against protein antigens of the bacterial cell wall, rather than increased recognition against lipopolysaccharide. In another study conducted by our laboratory group, cows administered 6 doses of J5 E coli bacterin responded with higher serum IgG2 concentrations, compared with concentrations in cows administered 3 doses, at 30 days after the fourth, fifth, and sixth doses. This pattern was also evident in the study reported here, although differences between cows administered 5 doses and 3 doses were not evident until 28 days after the fifth dose.

Administration of the J5 E coli bacterin in an array of anatomic sites resulted in higher serum antibody concentrations than those in cows administered the bacterin at only 1 anatomic location. This was especially notable for the IgG1 concentration, in which cows in the 5XSR group consistently had higher concentrations than those in all other groups starting 28 days after the third vaccination. Although differences in the IgG2 concentration between the 5XSR group and the other groups were not as pronounced, changes in location of injection sites resulted in a more protracted antibody response.

Serum J5 E coli–specific immunoglobulin concentration decreased in the 3X and 5XN groups, but not in the 5XSR group, to the same concentration as in unvaccinated cows by 84 days after the fifth vaccination. In this study, we did not assess whether an increase in antibody response would manifest as a lower incidence of clinical coliform mastitis. However, our laboratory group determined in another study that cows may have a period of increased incidence of severe coliform mastitis near peak lactation (60 to 150 days of lactation) despite administration of 3-dose regimens of J5 E coli bacterin. This suggests that a standard vaccination protocol for nonlactating cows during the periparturient period may not offer optimal protection much beyond 50 days after vaccine administration. This was corroborated in a study conducted in New York in which the investigators determined that cows vaccinated against J5 E coli had an increase in adjusted daily milk production for 21 days after clinical mastitis, compared with results for unvaccinated control cows; however, the protective effect of vaccination waned with increasing number of days of lactation at onset of the clinical mastitis. Thus, changing the location of injection sites in dairy cows may improve the duration of the immune response, which has been a major limitation of protection resulting from use of J5 E coli bacterins.

This is consistent with the concept that naïve lymphocytes travel through the blood and lymphatic systems searching for antigen-presenting cells that express antigen-receptor complexes that match the lymphocyte antigen receptor. Multiple injection sites may allow for migration of antigen-presenting cells to a more diverse number of lymphoid centers, thus increasing the probability of complimentary interactions between antigen-presenting cells and lymphocytes.

To our knowledge, the study reported here is unique in comparing results for a vaccination regimen that involved the use of sequential injection sites as opposed to use of 1 anatomic site. Our results are in agreement with those of another study in which investigators also reported that a J5 E coli vaccination regimen that included S. cerevisiae as well as intramammary administrations resulted in enhancement of anti-J5 E coli immunoglobulin titers in milk and serum. Thus, the study reported here offers a potentially new variable to be considered when assessing antibody response to bacterins. The relatively small size of this study suggests that further investigation is required, and the effect of sequential changes in injection sites on results for other vaccination regimens, especially those not requiring multiple doses or those that involve the use of nonbacterin antigens (such as toxoids or viruses), is speculative.

Sequential changes in location of injection sites during administration of a 5-dose regimen of J5 E coli bacterin to mature lactating dairy cattle resulted in increased serum anti-J5 E coli IgG1 and IgG2 concentrations. For dairy producers that use J5 E coli bacterins, and especially those that administer them in accordance with hyperimmunization protocols, administration at multiple anatomic sites may improve the antibody response relative to that for protocols in which only 1 anatomic site is used. However, the protective effect that the increased antibody response may have on the incidence of clinical coliform mastitis is unknown.

References


