Milk and Serum J5-Specific Antibody Responses, Milk Production Change and Clinical Effects Following Intramammary *Escherichia coli* Challenge For J5 Vaccinate and Control Cows

J5 ANTIBODY IN COWS FOLLOWING *E. coli* CHALLENGE

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Holstein dairy cows (4 J5 vaccinates, 4 controls) selected for no recorded intramammary disease and low somatic cell count (SCC) during the previous lactation were challenged by intramammary infusion of *Escherichia coli*. Vaccination with J5 was at -8 wk and again -4 wk from anticipated calving date. Cows were challenged at 8 - 16 days in milk (DIM). Shedding of *E. coli* in milk was significantly higher among controls than vaccinates (no shedding) from 6 h to 21 h post-challenge. From 21 h to 132 h post-challenge, SCC in challenged quarters of controls (5,429,000/ml) was significantly higher than that of vaccinates (490,000/ml). On the day after challenge, control cows lost 8 kg of milk production, while vaccinates gained 0.5 kg, a significant difference. In serum immediately prior to challenge, J5-specific IgG1 was significantly higher, IgG2 was nearly significantly higher, and IgM was the same in J5 vaccinates relative to controls. Vaccinates had proportionally more IgG2 in serum post-calving and in the first 12 h following challenge, and less IgG2 in milk 24 h after challenge than controls, approaching statistical significance. The ratio of J5-specific IgG1 and IgG2 combined compared to IgM was significantly higher in vaccinates than controls in pre-challenge serum (15.8, 3.2 ratios respectively) and milk (5.0, 1.3). Cows with higher IgM titers in milk 12 h post-challenge lost significantly more milk. Vaccination with J5 was significantly associated with higher production of J5-specific IgG1 and IgG2 in early lactation, reduced SCC, faster clearance of *E. coli* from milk, and less milk production loss following intramammary challenge.

Bovine coliform mastitis is most commonly caused by *E. coli* and *Klebsiella* spp. (16, 21, 30, 32). Coliform mastitis can cause abnormal milk, milk production
loss, treatment costs, and death of cattle (20, 21, 30).

Vaccination against coliform mastitis with J5 bacterins has been used in the dairy industry for more than 15 yr (10, 15). However, the effects of J5 immunization on the bovine mammary immune response have not yet been fully explained (3, 12). It is not clear whether J5 vaccination results in J5 *E. coli*-specific IgM, IgG1, or IgG2 antibody production, changes in their relative proportions produced in milk or serum, or whether such changes are associated with resistance to coliform mastitis (2, 5, 17, 19, 28, 32). It is generally accepted that either IgM, IgG2, or both isotypes are particularly important in opsonization of bacteria for PMN phagocytosis, and increases in these antibodies against the target mastitis pathogens are desired goals of vaccination against mastitis (2, 5).

The primary objective was to evaluate a commercial J5 vaccine for protection against *E. coli* challenge, and to statistically test for associations between J5 vaccination, outcome measures of clinical mastitis (CM) severity, and J5-specific IgG1, IgG2, IgM antibodies in milk and serum before and after challenge.

**MATERIALS AND METHODS**

**Experimental design and timeline.** Pregnant Holstein cows selected to be J5 vaccinates (n = 4) were vaccinated with J5 bacterin (J-VAC®, Merial Ltd, Duluth, GA) at –8 wk from when they were due to calve (at dryoff) and again –4 wk from due date (mid dry period). Vaccine (2 ml) was administered subcutaneously in the supramammary lymph node region. Pregnant controls (n = 4) were not given a sham immunization. Milk samples were collected immediately prior to intramammary
infusion challenge (approximately 2 wk after calving) with *E. coli* O:157 and again 12 h and 24 h post-challenge. Blood samples were collected – 4 wk from calving due date, post-calving, immediately prior to intramammary challenge, and 12 h and 24 h post-challenge.

**Selection of cows for study.** Holstein dairy cows (n = 8) were purchased from a commercial dairy. All cows had completed at least one previous lactation, were in late lactation, had no recorded cases of disease in the previous lactation, all monthly Dairy Herd Improvement Association (DHIA) somatic cell count (SCC) tests < 250,000/ml, and similar milk production for the previous lactation. Milk production of the 8 cows was approximately 10,900 kg per 305 d in the previous lactation. The first 4 cows that calved were controls, and the last 4 cows that calved were J5 vaccinates.

**Milking, SCC, bacteriology and selection of challenge quarters.** As cows reached approximately 14 d before their calving due date, they were transported to a research tiestall facility at Cornell University. All 8 cows calved uneventfully, on or a few days before or after their due date, with live calves and no dystocia.

Cows were milked twice daily using a bucket milking system within industry mechanical performance standards. Chlorhexidine udder wash, predip and postdip with 0.5% chlorhexidine teat dip, and forestripping were utilized. Until 3 d before challenge, cows were milked in the typical way, with milk of all 4 quarters harvested and weighed in the same bucket. Beginning 3 d before challenge, each quarter was milked separately and the quarter’s milk was weighed separately until the intramammary challenge. From the milking following challenge until 7 d post-
challenge, the challenged quarter and the contralateral quarter were milked and weighed separately, and the other 2 quarters were milked together and their combined milk weighed separately (e.g. if RF quarter was challenged, LF quarter was also milked separately and the RR and LR quarter were milked and weighed together).

Milk samples were tested for SCC at 7d, 2d, and 1d before challenge from all 4 quarters, and duplicate milk samples collected using aseptic technique were cultured for bacteria, including *Mycoplasma* spp., at 7d and 2d pre-challenge. Milk samples for SCC enumeration were transported to the nearby DHIA laboratory and were measured using a Fossomatic (Foss in North America, Eden Prairie, MN) cell counter; samples too thick to go through the Fossomatic were tested by Direct Microscopic SCC. Samples for bacteriological culture were transported cold to the Quality Milk Production Services (QMPS) Central Laboratory at Cornell University for microbiological culture according to protocols recommended by the National Mastitis Council (18). Contamination was defined as the isolation of more than 3 types of bacteria other than *Staphylococcus aureus*.

Challenge (and contralateral) quarters were selected based upon consistency of milk production, having no major pathogens isolated from milk cultures, and SCC < 100,000/ml.

**Intramammary *E. coli* challenge.** Cows were challenged two at a time on the same day of the week, between 7 and 14 d following their calving due dates, over a period of 35 d. Immediately following the pm milking on the day of challenge, the chosen quarter was infused with approximately 1000 cfu of *E. coli* intramammary (50 cfu/ml × 20 ml in sterile saline). Each challenged quarter was aseptically sampled for
milk culture immediately before the challenge infusion. A remnant of the 20 ml intramammary challenge solution was transported cold immediately back to the laboratory at QMPS for confirmatory counting of the cfu/ml of E. coli. The challenge strain of bacteria was an E. coli O:157 strain\(^b\) originally isolated from a clinical mastitis case. This strain had been used for challenge experiments previously (22).

Rectal temperature and clinical signs were monitored for 24 h post challenge. Cows’ behavior was observed several times per day for the remainder of the trial. Milk samples were collected for SCC and culture from the challenged quarter every 3 h for first 24 h post-challenge, then every 12 h until 180 h post-challenge. Challenged quarter, contralateral quarter, and the other 2 quarters’ combined milk production were monitored as described earlier for the rest of the trial. Cows were sold 8 d post-challenge.

\(^b\) Available from Quality Milk Production Services, Cornell University
**J5-specific antibody.** Blood samples for measurement of serum J5-specific antibodies (IgM, IgG1, and IgG2) were collected -4 wk from calving and within 4 h following calving, and both milk and blood samples were collected immediately prior to challenge, and 12 h and 24 h post-challenge. No blood samples were collected -8 wk from calving because based on preliminary data from one of the authors (JB), we did not expect much seroconversion after only the first vaccination. Blood samples were centrifuged (750 × g, 15 min, 5°C), and the serum was stored at –80°C in 5 × 1-ml aliquots in microtubes. Milk was not centrifuged and milk samples were stored at –80°C in 5 × 1-ml aliquots in microtubes. Antibody was determined by ELISA as described by others (6).

Briefly, flat bottom 96-well ELISA plates (BD Falcon, Bedford, MA) were coated with 100 ul of J5 *E. coli* (1 × 10⁹ cfu/ml in sterile saline) as the target antigen (killed with 1% phenol), covered with an adhesive plate sealer and left on a flat surface at room temperature for 12 h to enable adherence of the bacteria to the plate wells. There were 4 negative control wells with no *E. coli* added on each plate to assess non-specific binding of antibodies to plastic. To perform the assay, plates were washed 3 times with a wash solution (0.1% Tween 20 in aqueous normal 0.9% saline) that functioned as a protein blocker, and various control and test samples were added to appropriate wells. Four wells of each plate received a 1:400 dilution of low immunoglobulin fetal bovine serum as the assay negative control. An additional 4 wells received a 1:400 dilution of serum from a 6 times J5 immunized dairy cow as the assay positive control. Any plate for which the standard deviation of the mean O.D. of this positive control was more than 1.0 was repeated on a different assay day. Finally, additional control wells included 2 blanks (no *E. coli* or other test reagents) against which the plate reader was blanked, 2 wells with no *E. coli* or test serum but with all
other test reagents added, 2 wells with *E. coli* but no other test reagents except serum, and 2 wells with *E. coli* plus all other test reagents except serum. The remaining 80 wells per plate received test milk or sera as described below after receiving 125 μl of diluent (10% 10x PBS [pH 7.3], 0.05% Tween 20 in sterile water) as the solution for serial dilutions of these test samples.

Serum test samples for detection of J5-specific IgG1 were plated in 30 serial (doubling) dilutions from 1:2 to 1:1,073,741,824. Serum test samples for detection of J5-specific IgG2 and J5-specific IgM were plated in 20 doubling dilutions from 1:2 to 1:1,048,576. Whole milk was used for detection of J5-specific IgG1, IgG2 and IgM; all samples were plated in 15 doubling dilutions from 1:2 to 1:32,768. These ranges of dilutions were sufficient so that all milk or serum samples for each class of antibody were eventually diluted to an optical density (O.D.) reading of < 0.100, which was the OD of the fetal bovine serum negative control and thus used as an indication of endpoint titer. Therefore, the highest dilutions of test samples that resulted in O.D. < 0.100 were recorded as the titers.

**Statistical analyses.** Shedding of *E. coli* in milk (cfu/ml) was tested for differences between controls and J5 vaccinates with mixed linear models and least-squares means (Statistical Analysis System, Cary, NC [SAS], PROC MIXED). Differences between controls and vaccinates in SCC/ml in milk, mean daily milk production change from the 8 d before vs. the 7 d after challenge, and J5-specific antibody titers preceding and following challenge were tested with ANOVA (PROC ANOVA). Ratios of IgG1:IgG2, and IgG1 and IgG2 to IgM were evaluated using linear regression with general linear models (PROC GLM). Linear regression was also used to evaluate other possible explanatory variables associated with shedding of
E. coli in milk 3 h post-challenge, and with differences in milk production between controls and vaccinates following challenge. Quarter milk production change was similar to total cow milk loss; therefore only the latter was analyzed because of its practical importance. A mixed linear model with least-squares means (PROC MIXED) was developed to explain variation in total cow milk production on a given day of lactation, whether before or after the challenge. Repeated measures of the same cow were taken into account using a random cow effect. The daily milk production models included the effects of DIM on total cow milk production. The ratios of IgG1:IgG2 and IgG1 and IgG2 (combined) to IgM were also tested as potential explanatory variables (covariates) for each of the models examining variation among cows in outcomes following mastitis. All statistical analyses were performed using SAS 8.2.

For example, evaluation of J5-specific antibody response at a given time, specific isotype, and in milk or serum used ANOVA (PROC ANOVA):

\[ Y = J5 \text{ Vacc} + e \]

Expanded ANOVA was:

\[ Y = J5 \text{ Vacc} + \text{LACT} + e \]

Where \( Y \) = Serum titer of IgG2 24 h post-challenge, \( J5 \text{ Vacc} = J5 \) vaccinate (1) or control (0), \( \text{LACT} \) = Lactation number 2, 3, or 4, and \( e \) = unexplained variation.

**RESULTS**

**Bacteriology and SCC before intramammary challenge.** Challenged quarters had no major pathogens isolated before the challenge. Up until immediately prior to challenge, all 16 challenged and contralateral quarters had consistent milk production. Mean pre-challenge SCC was 45,000/ml in challenged quarters, ranging
Clinical response and follow up culture of challenge solution after intramammary challenge. Cows were challenged at a median of 13 DIM (range 8 - 16 DIM). There were no clinical signs of mastitis after challenge, and except for one control cow with rectal temp of 104.5°C at 12 h post-challenge, all rectal temperatures at 12 h and 24 h post-challenge were normal. All cows had normal appetite following challenge. Cultures of the remnants of all 8 challenge infusion solutions revealed *E. coli* between 600 and 1700 cfu/20 ml, all within one log of the target of 1000 cfu *E. coli*/20 ml.

Shedding of *E. coli* in milk after intramammary challenge. Shedding of *E. coli* in milk was significantly higher among controls than vaccinates between 6 h and 21 h post-challenge (all P < 0.05, mixed linear model, PROC MIXED). During that time, cfu/ml of *E. coli* ranged between 195 and 5500 for 3 of the 4 control cows. Of the 4 vaccinates, one cow shed 10 cfu/ml and another cow shed 20 cfu/ml of *E. coli* at 3 h post-challenge, otherwise the 4 vaccinates shed no bacteria from 3 to 21 h post-challenge. From 24 h post-challenge on, no statistically significant differences were observed between vaccinates and controls.

Because most of the shedding of *E. coli* in milk among J5 vaccinates took place at 3 h post-challenge, that time point was investigated using a general linear model. Vaccination with J5 was associated with less shedding of *E. coli* in milk (P < 0.0001, linear regression, PROC GLM). Within only the control cow population, serum J5-specific IgM antibody just prior to challenge was associated with reduced *E. coli* in milk. 
coli shedding at 3 h post-challenge (significant interaction term, P < 0.0001). This model for shedding of E. coli in milk at 3 h post-challenge was highly explanatory (R^2 = 0.99, P < 0.0001, PROC GLM).

**SCC in milk after intramammary challenge.** At 21 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, 108 h, and 132 h following challenge, SCC in challenged quarter milk was significantly higher for control cows than for vaccinates. Most of the controls’ challenged quarter SCC/ml values were between 1,805,000/ml and 14,559,000/ml, with mean of 5,429,000/ml while the vaccinates’ challenged quarter SCC/ml were mainly between 81,000/ml and 373,000/ml, with mean of 490,000/ml (all P < 0.05, ANOVA, Fig. 1).

**Milk production change after intramammary challenge.** Control cows showed a significant reduction in milk yield on the day after challenge (-7.7 kg) compared to essentially no change by vaccinated cows (+ 0.5 kg) (P < 0.02, mixed linear model, PROC MIXED). Thereafter, milk production remained lower in control cows when compared to vaccinated cows, but was not significantly different due to the relatively small study size and the large between-cow variation (Fig. 2).

**J5-specific antibodies in milk and serum.** Negative control and blank wells all had O.D. <0.100. Titers for the 3 classes of J5-specific antibody were not significantly different in serum -4 wk from calving (just preceding the second and last immunization), in serum immediately following calving (approximately 4 wk after the last J5 immunization) or in milk immediately prior to intramammary challenge (at 8-16 DIM), between vaccinates and controls (all P ≥ 0.11, ANOVA).
However, immediately prior to challenge serum J5-specific IgG1 antibody was significantly higher for J5 vaccinates (mean titer 1:3584) than controls (mean titer 1:1024) (P = 0.003, ANOVA, Fig. 3). Serum J5-specific IgG2 antibody was also higher for J5 vaccinates (mean titer 1:1088) than controls (mean titer 1:240) prior to challenge; the difference approached statistical significance (P = 0.07, ANOVA, Fig. 3). Serum J5-specific IgM antibody was not significantly different between J5 vaccinates (mean titer 1:320) and controls (mean titer 1:448) prior to challenge (P = 0.21, ANOVA, not shown).

By 12 h post-challenge, J5-specific serum IgM response in controls (mean titer = 1:576) was higher than that for vaccinates (mean titer =1:200), approaching statistical significance (P = 0.07, ANOVA). J5-specific IgG1 and IgG2 in serum and all 3 antibody classes in milk were not significantly different among the treatment groups at 12 h post-challenge (all P > 0.22, ANOVA). At 24 h post-challenge, all 3 classes of J5-specific antibody were not different in milk or serum between vaccinates and controls (all P > 0.11, ANOVA).

**Ratios of J5-specific antibody isotypes.** There were differences in IgG1:IgG2 ratios approaching statistical significance between J5 vaccinates and controls as follows: post-calving serum ratios 0.6 in vaccinates, 1.9 in controls (P= 0.15, Type 3 SS F test, linear regression); 12 h post-challenge serum ratios 3.3 in vaccinates, 17.0 in controls (P= 0.16); 24 h post-challenge milk ratios 404.0 in vaccinates, 44.0 in controls (P= 0.18). There was a trend that vaccinates had proportionally more IgG2 in serum and less IgG2 in milk than controls.

The ratio of J5-specific IgG1 and IgG2 combined to IgM (IgG’s:IgM) was
investigated. Milk and serum of J5 vaccinates were significantly higher in J5-specific IgG1 plus IgG2 relative to J5-specific IgM than that of control cows immediately pre-challenge (Fig. 4a). The ratios of IgG’s:IgM for J5 vaccinates and controls, respectively were: -4 wk from calving serum 2.4, 2.4 (P = 0.99, ANOVA); post-calving serum 6.1, 0.5 (P = 0.10); pre-challenge serum 15.8, 3.2 (P = 0.02); pre-challenge milk 5.0, 1.3 (P = 0.03) (Fig. 4a); 12 h post-challenge serum 51.5, 7.6 (P = 0.27); 12 h post-challenge milk 76.8, 66.0 (P = 0.91) (Fig. 4b); 24 h post-challenge serum 15.3, 7.6 (P = 0.18); 24 h post-challenge milk 306.7, 35.4 (P = 0.31, all tests by ANOVA) (Fig. 4c). This indicated that J5 vaccinated cows’ immune response was more toward J5-specific IgG1 and IgG2 antibodies while that of controls had proportionally more J5-specific IgM antibody, especially just before challenge. Both the IgG1 to IgM ratio and IgG2 to IgM ratio had similar results (data not shown).

Milk production change and J5-specific IgM. Diagnostic plots (including residual analysis) for the milk production linear model revealed that one cow was driving the model as an outlier. Therefore that cow was excluded (she was excluded only from this analysis of milk production change), which resulted in a better fitting model according to diagnostic plots. The resultant model (for difference in mean daily milk production for the 8d pre-challenge compared with the 7d post-challenge) was highly explanatory ($R^2 = 0.78$, $P = 0.009$, linear regression, PROC GLM). Cows having relatively high milk IgM antibodies against J5 by 12 h post-challenge were significantly more likely to have greater milk production loss following the E. coli challenge.

A linear model for milk production change (with all 8 cows included) following challenge showed strong trends but was not significant ($R^2 = 0.50$, $P = 0.18$)
linear regression, PROC GLM, Table 1). Cows that were J5 vaccinates and that had higher J-5 specific IgG1:IgG2 ratio in serum after calving had less milk production loss following CM (Table 1). The two effects were only marginally statistically significant (P= 0.10, 0.11, respectively).

DISCUSSION

J5 efficacy. Vaccination with this J5 bacterin conferred protection following intramammary infusion challenge with *E. coli*. Vaccination was associated with almost immediate clearance of bacteria from milk, while controls shed *E. coli* in their milk for approximately 24 h. The SCC in milk of vaccinates was approximately 10% of SCC in controls following challenge, less than 500,000/ml mean for vaccinates and over 5,000,000/ml for control cows. Reduced SCC is an important indicator of reduced mammary inflammation and less milk loss following clinical mastitis, and is an important test of milk quality in the dairy industry (11, 25). After 60 h post-challenge, the vaccinates had SCC < 300,000/ml and controls were between 1,000,000 and 2,000,000/ml. Vaccinates had less milk production loss following challenge than controls by more than 3.0 kg per day, although this was statistically significant for only one d post-challenge.

J5 vaccination and production of J5-specific antibodies. Both controls and vaccinates had some J5-specific antibody of all 3 classes in serum and milk at all stages of the experiment. This suggests that all cows had some previous exposure to coliform mastitis pathogens sufficient to mount some immune response, including memory type responses. Considering that coliforms are widespread in dairy cow environments, this is reasonable (21, 32).
Immediately prior to challenge at approximately 2 wk into lactation, and 6 wk after the second of 2 vaccinations, serum *E. coli* J5-specific IgG1 antibody was significantly higher in J5 vaccinates than controls, and serum IgG2 was also higher in vaccinates at borderline statistical significance. The proportion of IgG1 and IgG2 (compared separately or combined) to IgM was significantly higher among J5 vaccinates immediately before challenge, 5 times higher in serum and 4 times higher in milk.

Thus the controls had relatively more IgM antibody to *E. coli* J5 just prior to intramammary infusion challenge, while J5 vaccinates had more IgG1 and IgG2 antibody. Exposure of B cells to antigen within a given host and a particular cytokine milieu stimulates a class switch from production of IgM to IgG1, IgG2 or other isotypes. A key component of effective host defense and immunological memory is this irreversible B cell genetic change from IgM to production of other antibody isotypes including IgG1 and IgG2 (5, 14). Originally described in mice, an antibody response in other species including the bovine featuring proportionally more production of IgG2 antibody has been called part of a type 1 response, while a response with more IgG1 is part of a type 2 response (4, 13, 27).

It has been suggested that an IgG2 type 1 response should be beneficial against bovine mastitis because IgG2 is an important opsonizing antibody aiding in neutrophil phagocytosis of bacteria, and IgG2 has the ability to readily fix complement (1, 5, 12, 24). In milk, the most important opsonizing antibody against coliform bacteria is IgG2, especially early in the infection (5, 7). Phagocytosis by PMN and the associated clearance of coliform bacteria from the mammary gland has been reported as optimal.
when IgG2 increases within 4 hr following intramammary infection, 6 to 12 h before the greatest influx of PMN from blood to milk (5, 23). The availability of more IgG2 against J5 immediately after bacteria enter the mammary gland appears to be an important benefit of J5 vaccination; because of previous natural exposure, control cows produced a similar response but only 12 hr after infusion of E. coli. The immune response of dairy cows in early lactation has a type 2 bias, while later in lactation the bias is type 1, which may be associated with greater protection against mastitis (26). These present results suggest that increased production of both J5-specific IgG1 and IgG2 are important mechanisms of J5 vaccine protection, including production of a higher proportion of IgG2 (lower IgG1:IgG2 ratio) than in control cows. In fact, the post-calving serum ratio of IgG1:IgG2 was less than one (0.6) in vaccinates, the only time in all of the data points where the immune response showed higher J5-specific IgG2 than IgG1, a type 1 response.

It has been previously speculated that increased production of J5-specific IgG2 could be a key mechanism of protection by J5 immunization in cattle (2, 5, 12, 28). Calves were reported more resistant to pneumonia caused by Haemophilus somnus in association with higher IgG2 antibody specific for H. somnus, while other antibody isotypes were not protective (8, 9). However, it has also been reported that while J5 vaccinates had higher IgG1 and IgG2 following vaccination as well as following clinical mastitis, these antibodies were not considered protective based on clinical mastitis signs (17). The present study demonstrated reduced milk loss, bacterial shedding and SCC following mastitis, together with higher IgG1 and IgG2 response among J5 vaccinates, especially in the first 12 hr after bacteria entered the gland.

In a previous study, antibody responses of 136 Holsteins in 3 dairy herds (48
first lactation cows, 88 older cows) were studied following vaccination with ovalbumin and J5. It was concluded that there are many sources of variation in CM among herds, and that herds should probably be evaluated separately for J5 effects on antibody and associations with CM (29). The current study used cows that originated from one commercial dairy herd, so the herd effect could not be studied. Conversely, there is some benefit from this in that there was no herd effect to possibly affect results between cows.

From the present study as well as some previous reports, it seems that J5 vaccination is associated with a memory antibody response of IgG1 and IgG2 isotypes and that this is also associated with protection against mastitis. This leads to some important questions to be investigated further, such as what are the relevant antigen(s) in J5 bacterins? This has never been conclusively shown (3, 6, 12). Indeed, there is recent evidence that the most immunogenic antigen(s) in J5 bacterin are not LPS or Lipid A (6).

These results also raise the question of the optimum J5 immunization schedule for producing the most efficacious immune response. In a previous study of steers vaccinated with a J5 bacterin, 5 immunizations with J5 over 72 d were necessary for J5-specific IgG2 antibody to be significantly increased over preimmunization levels in serum. The authors suggested that multiple doses of J5 bacterin may be needed to result in high concentration of IgG2 reactive against J5 (6). Further investigations into the antigen(s) in J5 bacterin and the most cost-effective immunization schedule as measured by protection against naturally occurring mastitis cases in lactating dairy cattle are needed.
CONCLUSIONS

Vaccination with this J5 bacterin according to the described protocol was associated with faster clearance of *E. coli* from milk, reduced SCC, and less milk production loss following intramammary challenge. The vaccine was also associated with higher J5-specific IgG1 and IgG2 antibody in blood before challenge. While controls relied more on IgM antibody response to *E. coli* post-challenge, vaccinates produced an IgG1 and IgG2 immune response to *E. coli*, including a higher proportion of IgG2 (type 1 response). Vaccination with J5 appears to promote more IgG2 early in the course of intramammary infection, which is beneficial to phagocytosis by PMN and clearance of coliform bacteria from the mammary gland. Further investigations into the antigen(s) in J5 bacterin and the most cost-effective immunization schedule for dairy cattle are needed.

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REFERENCES


Figure 1
FIG. 1. SCC/ml in milk following intramammary infusion challenge among controls and J5 vaccinates. Significantly higher SCC among controls at 21 h, 36 h, 48 h, 60 h, 72 h, 84 h, 96 h, 108 h, and 132 h post-challenge (all P < 0.05, ANOVA).
Figure 2

Milk Production Before and After Challenge

Milk Production (Kg)

Days Relative to Challenge

- Controls
- Vaccinates

Figure 2
FIG. 2. Total cow milk production (kg) before and after intramammary infusion challenge of controls and J5 vaccinates. Significantly higher milk production among vaccinates for 1 d following challenge (P < 0.02, mixed linear model, PROC MIXED). From 2 through 7 d post-challenge, milk production was higher among vaccinates but not statistically different.
Pre Challenge Blood IgG1 and IgG2 and Milk Loss

Figure 3
FIG. 3. Change in (total cow) daily milk production (PRODDIFF in kg) following intramammary infusion challenge at 8-16 DIM among 4 controls (C) and 4 J5 vaccinates (V). Blood serum titers of J5-specific IgG1 and IgG2 antibodies (divided by 100) immediately prior to challenge. Serum IgG1 titers were significantly higher pre-challenge among vaccinates (P = 0.003, ANOVA). Mean serum IgG2 titers were higher pre-challenge among vaccinates at difference approaching statistical significance (P= 0.07, ANOVA). Other differences were not significant.
FIG. 4a. Mean titers of J-5 specific antibodies among J5 vaccinates and controls in serum and milk just prior to intramammary challenge infusion with *E. coli*. Significant differences were higher serum IgG1 pre-challenge (P = 0.003, ANOVA), higher serum IgG2 pre-challenge (borderline significant at P = 0.07), and higher IgG’s:IgM ratio in serum and milk (P = 0.02, 0.03, respectively) in J5 vaccinates than in control cows.
FIG. 4b. Mean titers of J-5 specific antibodies among J5 vaccinates and controls in serum and milk 12 h after intramammary challenge infusion with *E. coli*. Serum IgM was higher in controls than in J5 vaccinates (borderline significant at P = 0.07, ANOVA). All other antibody titers and ratios were not significantly different between controls and vaccinates.
FIG. 4c. J-5 specific antibodies in serum and milk 24 h after intramammary challenge infusion with *E. coli*. No significant differences between controls and vaccinates were observed.
TABLE 1. Linear regression model evaluating J5 vaccination and its association with change in daily milk production expressed as the difference from mean of the 8 days before intramammary *E. coli* challenge to the mean of the 7 days following challenge.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t value</th>
<th>Pr &gt; t</th>
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<td>BlCalvIg1to2</td>
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<td>1.43</td>
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</tr>
</tbody>
</table>

Quantitative effects of significant variables on milk production change

- Estimated effect on daily milk production change after challenge shown in kg
- BlCalvIg1to2= Ratio of titers for J5-specific IgG1 to IgG2 antibodies in blood serum just after calving
- Overall linear regression model $R^2 = 0.50$, $P = 0.18$