

Efficacy of an *Escherichia coli* J5 Bacterin Administered to Primigravid Heifers¹

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ABSTRACT

The efficacy of an *Escherichia coli* J5 bacterin for reducing the incidence of intramammary infections and clinical signs of mastitis was tested in first lactation heifers. Ten primigravid heifers were immunized with an *E. coli* J5 bacterin. Four heifers received a placebo. The bacterin and placebo were injected subcutaneously approximately 60 d prior to calving, 28 d later, and within 48 h after calving. Vaccinated and placebo-injected heifers were challenged by intramammary infusion of *E. coli* 727 in one mammary gland between 23 and 37 d after calving. All challenged quarters were diagnosed with an intramammary infection within 6 h after bacteria were infused. The severity and duration of local signs of clinical mastitis were reduced in vaccinated heifers compared with placebo-injected heifers. Systemic signs of clinical mastitis were limited and did not differ between treatment groups. Bacteria counts in milk from challenged quarters were lower in vaccinated heifers than in control heifers at 12, 15, and 48 h after challenge. Serum immunoglobulin G titers against whole-cell *E. coli* J5 antigen at calving were higher in vaccinated heifers than they were in controls. Vaccinated heifers had higher immunoglobulin G titers than did controls in mammary secretions at calving and immediately prior to challenge. Immunization of primigravid heifers with an *E. coli* J5 bacterin during the last trimester of gestation and at calving reduced the severity and duration of clinical signs following intramammary challenge with a heterologous strain of *E. coli*.

(**Key words:** mastitis, vaccine, *Escherichia coli* J5, heifers)

INTRODUCTION

Escherichia coli J5 vaccines have effectively reduced the incidence and severity of clinical mastitis in multiparous cows (2, 6). The protection offered by the vaccine was greatest for cows in their fourth or greater lactation (3). This result corresponded with the finding that older periparturient cows are the cows within a herd that are at the greatest risk for clinical mastitis caused by coliform bacteria (10). However, surveys within the last decade have indicated that the incidence of IMI and risk of clinical mastitis in heifers were previously underestimated (1, 8). Specifically, the risk of clinical coliform mastitis was comparable between multiparous cows and first lactation heifers (13). The economic impact of mastitis in primiparous heifers is potentially immense because first lactation heifers represent approximately 32% of the lactating cows in the United States (7). The high incidence of IMI in first lactation heifers at calving was attributed to the lack of hygiene and health management practices for heifers; however, these practices are afforded to lactating and dry cows (8). For example, experiments to determine efficacy of *E. coli* J5 vaccines established a protocol whereby immunizations were given at drying off, approximately 30 d later, and at the subsequent calving (2). Therefore, data are minimal on the impact of vaccinating primigravid heifers with *E. coli* J5 vaccines. The purpose of the current study was to determine the efficacy of an *E. coli* J5 bacterin administered during the last trimester of gestation and at calving in primigravid heifers following intramammary challenge with a heterologous strain of *E. coli*.

MATERIALS AND METHODS

Experimental Design

Fourteen primigravid heifers were assigned to four blocks of 3 and one block of 2. Two heifers within each block of 3 and both heifers in the block of 2 were vaccinated with an *E. coli* J5 bacterin (*Escherichia*

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Coli BACTERIN J5 strain; Pharmacia and Upjohn, Kalamazoo, MI). One heifer in each block of 3 was injected with a placebo containing adjuvant only. The treatment codes were not broken until data collection and analyses were completed. Heifers were immunized approximately 60 d prior to anticipated calving, 28 d later, and within 48 h after calving. Immunizations (5 ml) were subcutaneous on the upper part of the rib cage posterior to the scapula. Experimental heifers were housed and managed similarly.

Intramammary Bacterial Challenge

Escherichia coli 727, originally isolated from a naturally occurring IMI, was used as the intramammary challenge strain. Challenge inoculum was prepared as described by Hogan et al. (6). The right or left front mammary quarter of each experimental heifer was challenged by intramammary infusion of *E. coli* 727. The geometric mean of the colony-forming units for challenge inoculum was 67.6 (65 to 70 cfu) suspended in 1 ml of PBS. Heifers within a block were challenged on the same day. The geometric mean DIM during challenge was 30 d (23 to 37 d). Infusions were 4 h after the morning milking, and only uninfected quarters were infused.

Quarter Samples

Incidence of naturally occurring IMI during lactation was determined using quarter foremilk samples taken on d 0, 3, 7, 14, and 21 of lactation. Quarter foremilk samples also were collected 7, 5, and 3 d prior to bacterial challenge; immediately prior to challenge; and 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, 96, 168, and 336 h postchallenge. Sample collection and microbiological procedures were as previously described (10).

Colony-forming units per milliliter and SCC were determined in quarter foremilk samples during the postchallenge period. Colony-forming units were determined, and data were expressed as previously described (6). The SCC per milliliter of milk were determined by a Bentley Somacount 150 (Bentley Instruments, Inc., Chaska, MN). Samples from clinical quarters were diluted 1:10 and 1:50 (milk:PBS, vol/vol) for counting. Data were expressed as \log_{10} SCC/ml of milk.

Diagnoses of IMI and Clinical Signs

An IMI was diagnosed when bacteria were isolated from two consecutive samples. Duration of IMI was

the time (in hours) between the first and last isolation of bacteria from a quarter. The clinical status of all quarters was recorded on a five-point scale at the time quarter foremilk samples were obtained: 1 = normal milk and normal quarter, 2 = normal quarter but milk was questionable, 3 = normal quarter but abnormal milk, 4 = a swollen quarter and abnormal milk, and 5 = swollen quarter, abnormal milk, and systemic signs of infection. Rectal temperatures were measured immediately prior to challenge and each time quarter samples were collected postchallenge.

Milk Production and DMI

Milk production was measured electronically at each milking. Daily feed intake was recorded for all heifers. Postchallenge daily milk production and DMI were expressed as percentages of means for the 7 d prior to challenge $[(b/a) \times 100]$ where a = mean value for the 7 d prior to challenge, and b = daily value postchallenge].

Antibody Titers

An ELISA was used to determine antibody titer in serum and mammary secretions to *E. coli* J5. The ELISA procedures were essentially those detailed by Tyler et al. (13). *Escherichia coli* J5 were incubated for 18 h at 37°C. Heat-killed bacteria were coated onto microtiter wells by incubation overnight at 37°C. Isotypes were determined by rabbit anti-bovine IgG (Sigma Chemical Co., St. Louis, MO) and goat anti-bovine IgM (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Titer data were expressed as the reciprocal of the dilution \log_2 .

Statistical Analyses

Treatment differences between bacteria counts, rectal temperatures, SCC, DMI, milk production, duration of IMI, and clinical signs were tested by least squares ANOVA (9). Models included treatment, block, time of sampling, and second-order interactions between main effects. Relationships among IgG and IgM titers with bacteria counts in milk after challenge were tested by Pearson's correlation coefficient (11).

RESULTS AND DISCUSSION

Results of the current trial, in which heifers were vaccinated prepartum and at calving, were similar to those in which multiparous cows were tested using a similar challenge model (6). Immunization of primiparous heifers with an *E. coli* J5 bacterin in the

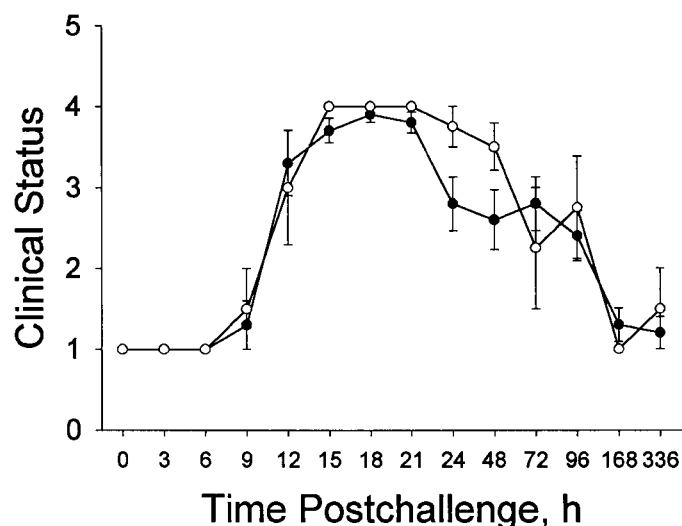


Figure 1. Mean clinical score following intramammary challenge with *Escherichia coli* 727 at 0 h in first lactation heifers immunized with *E. coli* J5 (●; n = 10) and in placebo-immunized controls (○; n = 4). Dispersion bars represent standard errors for each treatment at each sample time.

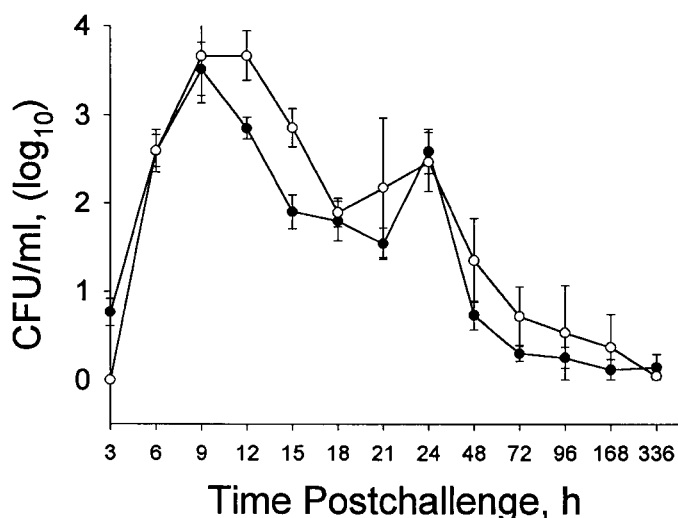


Figure 2. Bacteria counts in milk following intramammary challenge with *Escherichia coli* 727 at 0 h in first lactation heifers immunized with *E. coli* J5 (●; n = 10) and in placebo-immunized controls (○; n = 4). Dispersion bars represent standard errors for each treatment at each sample time.

current study did not prevent IMI but reduced the severity of clinical signs following intramammary challenge with a heterologous strain of *E. coli*. All quarters challenged were diagnosed with an IMI within 6 h after challenge. Geometric mean durations of IMI were 66.1 h for vaccinated heifers and 87.1 h for controls ($P < 0.05$). The mean clinical scores at 24 and 48 h after challenge were greater for controls compared with vaccinated heifers (Figure 1; $P < 0.05$). The geometric mean duration of local clinical signs including mammary swelling or abnormal milk were 93.3 h for controls and 44.7 for vaccinated heifers ($P < 0.05$).

Previous trials testing the efficacy of *E. coli* J5 bacterins in multiparous cows have demonstrated that vaccination reduced bacterial counts in milk from challenged quarters (5, 6). Similar results were observed in the current trial, which tested efficacy of an *E. coli* J5 bacterin in primiparous heifers (Figure 2). Bacterial counts in milk from challenged glands of vaccinated heifers were lower than those from control heifers at 12, 15, and 48 h after challenge ($P < 0.05$). Bacterial counts in milk peaked at 9 h after challenge in both groups, but a more rapid clearance of bacteria from the gland was observed in vaccinated heifers.

Protection against coliform mastitis afforded by vaccination with *E. coli* J5 bacterins has been ascribed to enhanced opsonization of bacteria and phagocytosis by neutrophils responding to the IMI (3). However, vaccination appears to have no effect

on the rate or magnitude of SCC response to intramammary challenge. Milk SCC did not differ between experimental groups in the present study (Figure 3) and previous trials (5, 6). Milk SCC responses typically were initiated 3 to 6 h after peak bacteria counts and reached the zenith 24 h after challenge. Milk SCC from vaccinated and control heifers

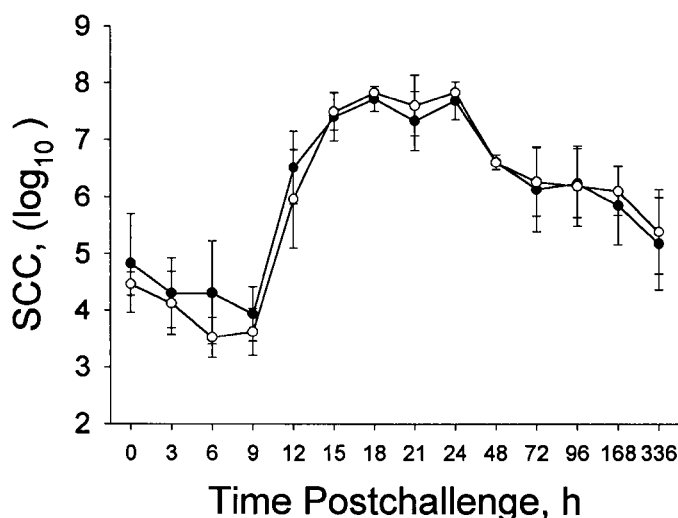


Figure 3. The SCC following intramammary challenge with *Escherichia coli* 727 at 0 h in first lactation heifers immunized with *E. coli* J5 (●; n = 10) and in placebo-immunized controls (○; n = 4). Dispersion bars represent standard errors for each treatment at each sample time.

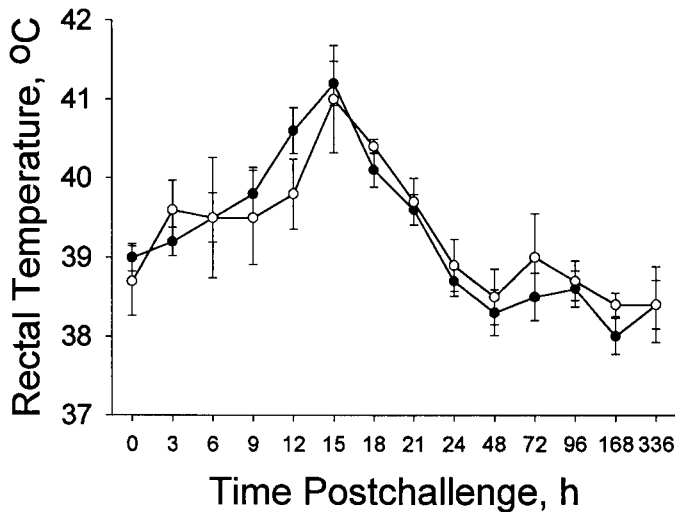


Figure 4. Rectal temperature following intramammary challenge with *Escherichia coli* 727 at 0 h in first lactation heifers immunized with *E. coli* J5 (●; n = 10) and in placebo-immunized controls (○; n = 4). Dispersion bars represent standard errors for each treatment at each sample time.

ers had returned to prechallenge concentrations by 14 d after challenge.

The bacterial strain used in the current trial, *E. coli* 727, has been repeatedly used as a challenge strain to induce clinical mastitis with limited systemic signs (6, 12). Systemic clinical signs again were limited following challenge with this strain. Vac-

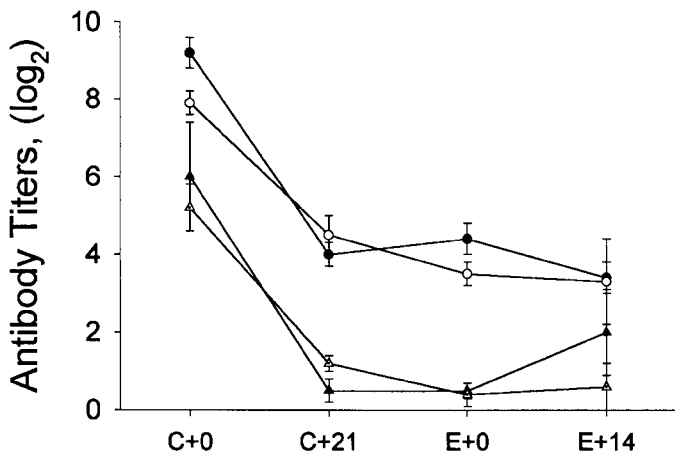


Figure 5. Mammary secretion antibody titers [IgG (●, ○); IgM (▲, △)] to *Escherichia coli* J5 in first lactation heifers immunized with an *E. coli* J5 bacterin (n = 10; closed symbols) and placebo-immunized controls (n = 4; open symbols). Samples were collected at calving (C + 0), on d 21 of lactation (C + 21), immediately prior to challenge (E + 0), and at 14 d after challenge (E + 14). Dispersion bars represent standard errors for each treatment at each sample time.

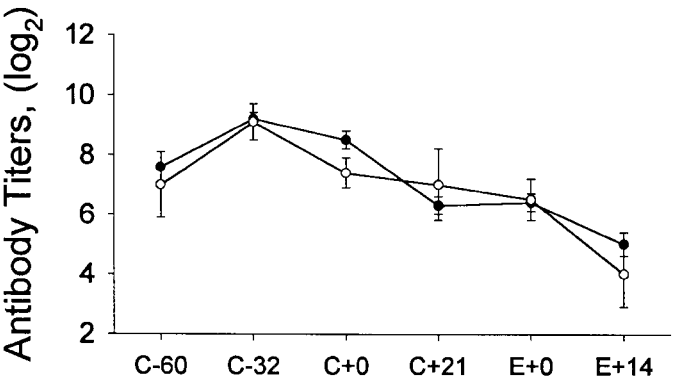
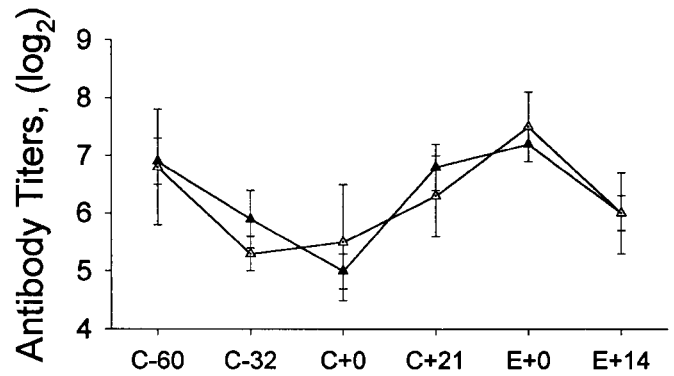


Figure 6. Serum antibody titers [IgG (●, ○); IgM (▲, △)] to *Escherichia coli* J5 in first lactation heifers immunized with an *E. coli* J5 bacterin (n = 10; closed symbols) and placebo-immunized controls (n = 4; open symbols). Samples were collected at 60 d prior to anticipated calving (C - 60), 28 d later (C - 32), calving (C + 0), on d 21 of lactation (C + 21), immediately prior to challenge (E + 0), and at 14 d after challenge (E + 14). Dispersion bars represent standard errors for each treatment at each sample time.

ination had no effect on systemic clinical signs including rectal temperature, milk production, and DMI ($P > 0.05$). Mean (\pm SE) peak rectal temperatures were $41.0 \pm 0.7^\circ\text{C}$ for control heifers and $41.2 \pm 0.6^\circ\text{C}$ for vaccinated heifers at 15 h after challenge (Figure 4). Rectal temperatures had returned to prechallenge values in both groups by 24 h after challenge. Milk production was reduced approximately 16% in controls and 23% in vaccinated heifers 1 d after challenge ($P > 0.05$). Milk production returned to prechallenge yields by d 2 in vaccinated and control heifers. Preceding the transient decrease in milk production, DMI decreased 12% the day control heifers were challenged. Dry matter intake was not altered by challenge in vaccinated heifers.

Titers for naturally occurring serum antibody specific for *E. coli* J5 were negatively associated with a risk for clinical coliform mastitis among cows within a herd (13). Active immunization of multiparous cows with whole-cell *E. coli* J5 increased titers

specific to conserved antigens (6). Titers responsive to immunization were negatively correlated with severity of clinical signs of mastitis following intramammary challenge (6). Immunization of primigravid heifers in the current study also enhanced IgG titers against whole-cell *E. coli* J5 antigen at calving. Immunoglobulin G titers in colostrum (Figure 5) and serum (Figure 6) collected at calving were greater ($P < 0.05$) in vaccinated heifers compared to controls. Milk collected from mammary glands of vaccinated heifers immediately prior to challenge had higher IgG titers than did milk collected prior to challenge from mammary glands of controls ($P < 0.05$). Immunization did not alter ($P > 0.05$) IgM titers against *E. coli* J5 in either serum (Figure 5) or mammary secretions (Figure 6).

The severity of clinical signs following intramammary infusion of *E. coli* were related to the peak bacterial count in milk from challenged quarters (5, 6). Enhancing mammary secretion IgG titers specific for *E. coli* J5 effectively reduced the peak bacteria counts in milk and subsequent clinical signs of mastitis (6). In the current trial, the correlation coefficient between IgG titers in milk from challenged quarters immediately prior to infusion and peak bacteria counts in milk from those quarters was -0.61 ($r^2 = 0.36$). Correlations were not significant among serum IgG or IgM titers and peak bacteria counts.

CONCLUSIONS

First lactation heifers represent a substantial population of animals within a dairy herd that is at risk for clinical coliform mastitis. Immunization of primigravid heifers during the last trimester of gestation and at calving with an *E. coli* J5 bacterin resulted in an enhanced humoral response and reduction in clinical coliform mastitis signs similar to that previously reported following immunization of multiparous cows.

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