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History and benefits of *Escherichia coli* J5 Mastitis vaccines

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Introduction

Considerable attention has been given to the protective effects of vaccinating laboratory animals, humans, and farm animals with the Rc rough mutant *Escherichia coli* (0111:B4) J5. *Escherichia coli* J5 lipopolysaccharide (LPS) lacks O-polysaccharide chains, thereby exposing the core antigens of LPS (2). The core antigens of LPS appear to be highly conserved among Gram-negative bacteria compared to the extremely heterogeneous O-polysaccharide antigens. Both passive and active immunization with *E. coli* J5 protected laboratory animals against endotoxic shock and bacteremia by heterologous strains (1, 11, 13). Vaccinating dairy cows with *E. coli* J5 bacterin during the dry period and early lactation significantly reduced the incidence of clinical mastitis during the first 90 d of lactation (3). A series of experiments were subsequently conducted to verify the efficacy of *E. coli* J5 for controlling Gram-negative bacterial mastitis and to determine the mode of action.

Intramammary challenge

***Escherichia coli* 487 Challenge.** A commercially prepared *E. coli* J5 bacterin was tested for efficacy in reducing intramammary infections and severity of clinical coliform mastitis in an experimental challenge trial (10). Ten cows were immunized at drying off, 30 days after drying off, and at calving. Ten control cows were not immunized. Right front quarters of all cows were infused with a heterologous strain of *E. coli* (strain 487) approximately 30 days after calving. *Escherichia coli* 487 was originally isolated from clinical mastitis and consistently results in acute clinical mastitis following intramammary challenge. Vaccinated cows had lower bacterial counts in milk and lower rectal temperatures than did unvaccinated controls following intramammary challenge. Milk production and dry matter intake were depressed greater in controls than in vaccinated cows. Milk SCC did not differ between experimental groups. Mean serum IgG titer to whole cell *E. coli* J5 was significantly greater in vaccinated than unvaccinated cows at 30 days after drying off, immediately prior to challenge and 7 days postchallenge. Milk IgG titer to *E. coli* J5 was greater at challenge in vaccinates compared with control cows. Vaccination with the *E. coli* J5 bacterin did not prevent intramammary infections, but did reduce severity of clinical signs following intramammary experimental challenge with a heterologous *E. coli* strain.

***Escherichia coli* 727 Challenge.** Efficacy of a commercially available *Escherichia coli* J5 bacterin was tested in an experimental challenge trial (6). Nineteen cows were vaccinated with an *E. coli* J5 bacterin, and 10 cows were injected with a placebo containing adjuvant only. Vaccine and placebo were administered at drying off, 30 days after drying off, and at calving. Cows were challenged approximately 30 days after calving by intramammary infusion with a smooth heterologous strain of *E. coli* (strain 727) previously shown to cause mild clinical mastitis. Vaccination with the J5 bacterin reduced duration of intramammary infections and local signs of clinical mastitis. Concentration of bovine serum albumin in milk 24 hours after challenge was greater in control cows than in J5-vaccinated cows. The SCC at 7 days postchallenge were greater in placebo-vaccinated cows than in J5-vaccinated cows. Bacterial counts were lower in placebo-vaccinated cows than in J5-vaccinated cows at 3, 6, and 9 hours postchallenge. In contrast, J5-vaccinated cows had lower bacterial counts at 2, 3, and 4 days postchallenge than did placebo-vaccinated cows. Systemic signs of clinical mastitis were relatively mild and similar between treatment groups. Rectal temperature, dry matter intake, and milk production did not differ between control and J5-vaccinated cows following challenge.

***Escherichia coli* J5 LPS Challenge.** The use of an *E. coli* J5 bacterin to reduce clinical signs of mastitis was tested in a LPS intramammary challenge model (7). Four cows were immunized at drying off, 30 days after drying off, and at calving. Four cows served as unvaccinated controls. A front mammary quarter was challenged by infusion of 10 µg of *E. coli* J5 LPS approximately 30 days after calving. Signs of clinical mastitis, including rectal temperature, depressed milk production, dry matter intake, and clinical severity score, did not differ between vaccinated and control cows following intramammary LPS challenge. Vaccination had no effect on mammary secretion IgM titers at calving, immediately prior to challenge, 8, 24, and 196 hours postchallenge. Colostrum IgG titers were greater in vaccinated cows than in control cows. Vaccination increased serum IgM titers at calving, immediately prior to challenge and 8 hours postchallenge. Serum IgG titers were greater in vaccinated cows than in negative controls 30 days into the dry period, calving, immediately prior to challenge, 8, 24, and 196 hours postchallenge. Serum and mammary secretion IgG and IgM titers were not correlated with clinical signs of mastitis.

Primigravid Heifers. 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 (4). 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 48h 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 6h 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 48h 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*.

Adjuvants. The effects of using a water soluble adjuvant or emulsifying the adjuvant with oil on the safety, antibody titer and clinical responses of an *Escherichia coli* J5 bacterin were tested in an experimental infection trial (5). Fifty-one cows were assigned to seventeen blocks of three. Two cows within each block of three were vaccinated with a commercially prepared *E. coli* J5 bacterin containing either a water soluble adjuvant or the same bacterin preparation emulsified in oil. One cow in each block was an unvaccinated control. Cows were immunized at drying off and 42 d later. The right or left front mammary quarter of each experimental cow was challenged by intramammary infusion of *Escherichia coli* 727 between 14 and 35 DIM. Areas of inflammation at the primary injection site were greater d 1, 2, and 3 following primary vaccination for bacterin containing oil-in-water adjuvant compared with bacterin containing water soluble adjuvant. Whey anti-*E. coli* J5 IgG titers were higher at calving for cows vaccinated with bacterin containing oil-in-water adjuvant than for cows either vaccinated with bacterin containing water soluble adjuvant or unvaccinated controls. Serum anti-*E. coli* J5 IgG titers were higher at calving for vaccinated cows than for unvaccinated controls. Peak bacterial counts in milk from challenged quarters were greater for unvaccinated controls than cows vaccinated with bacterin containing water-in-oil adjuvant. Bacterial counts in milk from challenged quarters and linical score both were greater in unvaccinated controls than cows vaccinated with bacterin containing water-in-oil adjuvant between 12 and 24 h post-challenge. Clinical responses were similar between unvaccinated controls and cows vaccinated with bacterin containing water soluble adjuvant.

Intramammary Immunization. Intramammary immunization was investigated as a procedure to reduce the clinical signs of coliform mastitis (12). Twenty-four cows were equally distributed to the following *Escherichia coli* J5 immunization schedules: 1) Subcutaneous injection 14 d prior to the end of lactation, intramammary immunization 7 d after drying off, and subcutaneous injection 30 d into the dry period; 2) subcutaneous injections at drying off, at 30 d into the dry period, and within 12h after calving; and 3) unimmunized controls. Intramammary immunizations were the infusion of vaccine via the teat canal into each of the four mammary glands. Cows were challenged by infusion of *E. coli* 727 into one uninfected mammary quarter at approximately 30 d after calving. Intramammary immunization enhanced antibody titers against *E. coli* J5 and *E. coli* 727 compared with subcutaneous immunization. Immunoglobulin G titers against *E. coli* J5 and *E. coli* 727 in whey were greater at the time of challenge and 7 d after challenge for cows that received the intramammary immunization than for cows immunized by only subcutaneous injections. Serum IgG titers against *E. coli* 727 were enhanced at 7 d after challenge for cows receiving intramammary immunizations compared with conventionally immunized cows. Serum IgM titers against *E. coli* 727 were higher at calving for cows receiving intramammary immunization compared with conventionally immunized cows. Immunization schedule had minimal effect on systemic and local signs of clinical mastitis following challenge.

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Opsonization

Six pairs of cows were used to determine the effects of immunization with an *E. coli* J5 bacterin on in vitro opsonization of *E. coli* 487 (9). One cow in each pair was either immunized with the vaccine or sham immunized at drying off, 30 days after drying off, and at calving. Opsonizing bacteria with serum collected from vaccinated cows 21 days after calving resulted in higher mean number of intracellular bacteria per phagocytosing neutrophil than opsonizing bacteria with serum collected from control cows. Phagocytic parameters using serum collected at drying off and calving did not differ between treatment groups. A trend for enhanced opsonic activity of colostrum from vaccinates was noted. Enhanced opsonization by serum from vaccinated cows coincided with higher serum IgM titer to *E. coli* J5 whole cell antigen compared to controls. Serum IgG titers to *E. coli* J5 did not differ between groups. Colostrum IgG titers to *E. coli* J5 were greater at calving in vaccinated than control cows. Both colostrum and milk collected 21 days after calving from vaccinated cows had higher IgM titers to *E. coli* J5 than did mammary secretions from control cows. Numbers of intracellular bacteria per phagocytizing neutrophil were correlated positively with IgM titers to *E. coli* J5 in both serum and colostrum.

Field trial

Efficacy of an *E. coli* J5 bacterin for preventing naturally occurring intramammary infections and clinical mastitis was tested in a 2.5 year field trial in a 225 cow commercial herd (8). Cows with odd number identification were vaccinated and cows with even number identification served as unvaccinated controls for each lactation during the study. Immunizations were subcutaneous on the upper part of the rib cage just posterior to the scapula at drying off, 30 days after drying off, and at calving. Percent quarters infected at calving with Gram-negative bacteria did not differ between treatment groups. Immunization with the *E. coli* J5 bacterin did not reduce prevalence of Gram-negative bacterial intramammary infections at calving, but did reduce incidence of clinical mastitis. The most striking difference between treatment groups was that 66.7% of IMI in controls became clinical during early lactation compared to 20% in vaccinates. Rate of Gram-negative bacterial clinical mastitis was 4.1-fold lower in vaccinated cows verses controls. Control and vaccinated cows also had similar percentage distributions of Gram-negative bacterial species within IMI diagnosed at calving. These data imply that the *E. coli* J5 vaccine did not prevent the occurrence of IMI, but did reduce the severity of the disease.

Conclusions

The efficacy of *E. coli* J5 bacterins was determined in a series of intramammary challenge and natural exposure trials. A consistent result of each infection trial was that the use of *E. coli* J5 bacterins did not prevent intramammary infections. However, the use of *E. coli* J5 bacterins reduced the severity and duration of mastitis. The primary means by which the bacterin reduces clinical severity appears to be related to opsonization and clearance of bacteria from the gland. Duration and severity of clinical signs were positively correlated with bacterial counts in milk following intramammary challenge with either virulent or avirulent strains. The primary opsonin was IgM, and IgG titers were not correlated with phagocytosis or reduction in clinical signs. Vaccination with an *E. coli* J5 bacterin had no effect on clinical response to intramammary infusion of LPS. The use of *E. coli* J5 bacterins during the dry period and early lactation reduced the incidence and severity of clinical mastitis during the first 90 days of lactation.

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